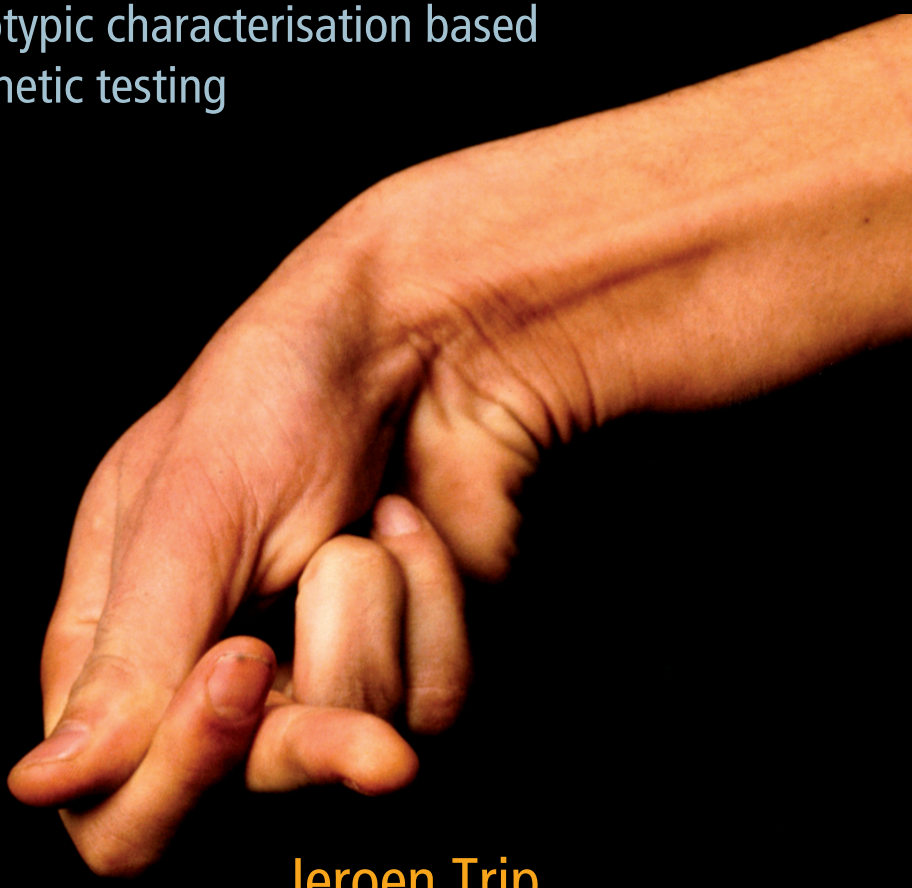
Redefining the non-dystrophic myotonic syndromes

Phenotypic characterisation based on genetic testing



Jeroen Trip

REDEFINING THE NON-DYSTROPHIC MYOTONIC SYNDROMES

Phenotypic characterisation based on genetic testing

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REDEFINING THE NON-DYSTROPHIC MYOTONIC SYNDROMES

Phenotypic characterisation based on genetic testing

een wetenschappelijke proeve op het gebied van de Medische Wetenschappen

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op gezag van de rector magnificus prof. mr. S.C.J.J. Kortmann,
volgens het besluit van het college van decanen
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Part I

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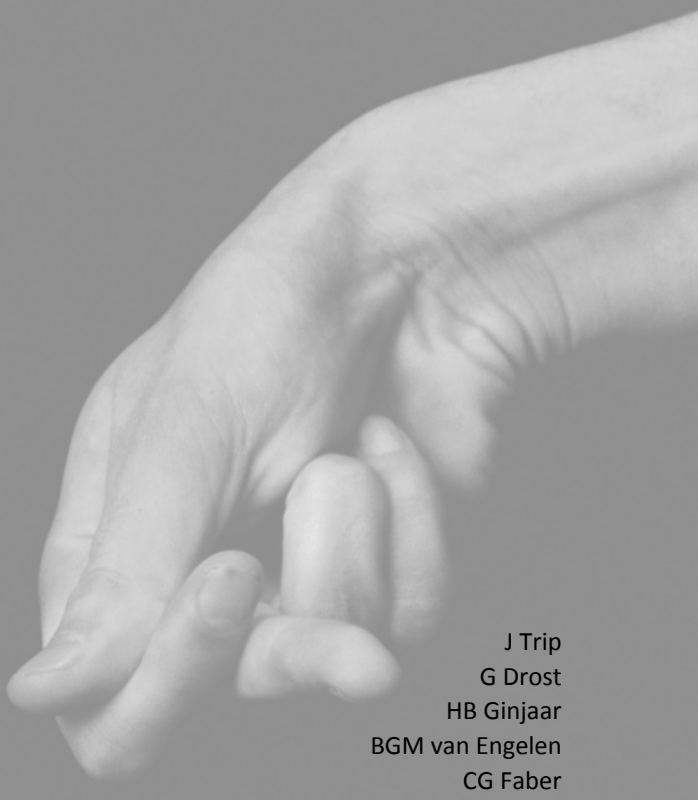
The non-dystrophic myotonic syndromes



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Adapted from Nederlands Tijdschrift voor Geneeskunde 2005;149:2093-2098

Non-dystrophic myotonic syndromes (NDMs) belong to the group of muscle disorders. The clinical approach to a patient with a suspected muscle disorder starts with a careful history and physical examination. Common presenting symptoms of a muscle disorder are negative muscle features like hypotonia, atrophy and muscle weakness. Examples of muscle diseases characterised by such negative features are the muscular dystrophies (e.g. dystrophinopathies), the congenital myopathies (e.g. nemaline and myotubular myopathy), and the inflammatory myopathies (among which polymyositis and dermatomyositis). Generally, less attention is paid to muscle diseases with positive muscle features like muscle cramps, involuntary muscle contractions, rippling muscles or myotonia, such as McArdle disease, rippling muscle disease and NDMs like myotonia congenita. Although myotonia is the main clinical feature in NDMs, several other clinical features can be distinguished (see Clinical box 1).

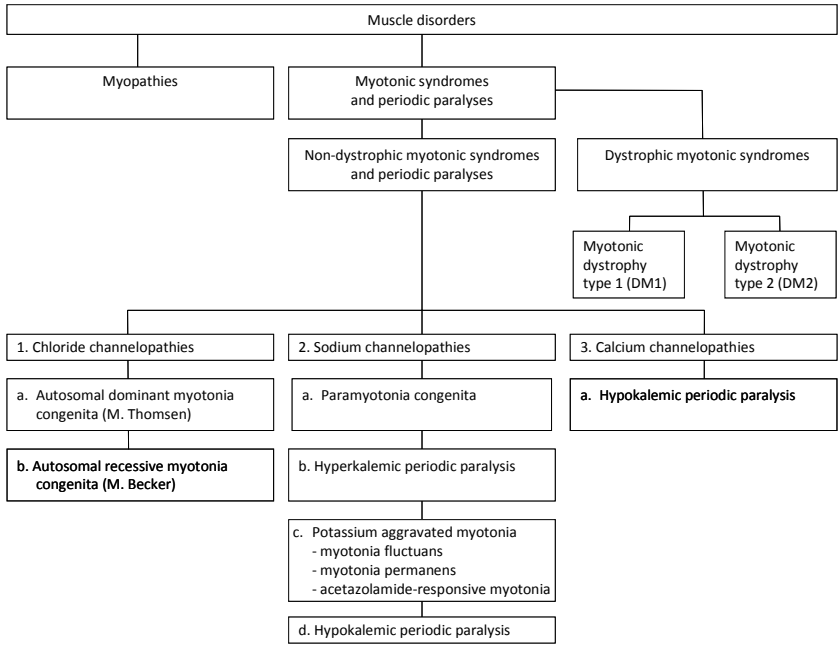

Figure 1.1a The skeletal muscle ion channelopathies, consisting of the non-dystrophic myotonic syndromes and periodic paralyses.

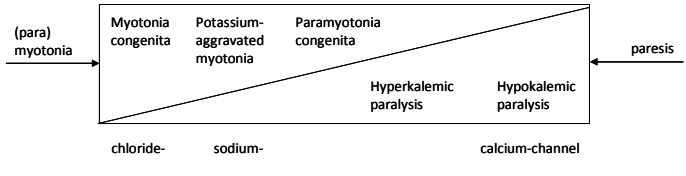

Figure 1.1b Overexcited and/or inactive: the spectrum of myotonia and paresis in skeletal muscle ion channelopathies.

CLINICAL BOX 1

Clinical features in non-dystrophic myotonic syndromes (NDMs)

Myotonia is a clinical phenomenon that refers to a delayed muscle relaxation after voluntary or evoked muscle contraction[1], which patients experience as muscle stiffness. It is a cardinal feature in myotonic disorders including NDMs.[2] A clinical examination of patients with a suspected NDM almost always reveals action myotonia and/or percussion myotonia. Both phenomena are most commonly and easily tested in the hand and arm muscles:

1. Action myotonia will be confirmed by a forceful grip after which the ability to relax the grip is abnormally delayed (Figure 1.2).
2. Percussion myotonia may be confirmed by mechanical stimulation: a tap with the percussion hammer on the thenar muscles, for example, will make these muscles contract for a prolonged period of time.

The first phenomenon may also be provoked in the eyelid muscles and both phenomena in leg muscles.

In NDM patients a voluntary muscle contraction may also provoke transient paresis, i.e., a transient decline in muscle force.[3] Myotonia as well as transient paresis may improve with continuing exercise, which is known as the warm-up phenomenon.[4,5] Both phenomena are best examined in the biceps muscle,[5] although the warm-up phenomenon may also be detectable in the eyelid muscles and both phenomena in the leg-muscles. Since both the warm-up phenomenon and the transient paresis may disappear after repetitive contractions, patients should be tested for these phenomena after a period of rest (e.g. after a 10 minute interval).

In contrast to the warm-up phenomenon, myotonia may also worsen after repetitive contractions, which phenomenon is called paramyotonia, a bastardisation of paradoxical myotonia.[6] In paramyotonia the muscle stiffness is already detectable after several contractions and subsequently worsens after additional repetitive contractions. Paramyotonia should, however, not be confused with “exercise-induced delayed-onset myotonia”, in which the myotonia only appears after a prolonged period of exercise.[7]

Figure 1.2 Myotonia of the right hand from a patient with recessive myotonia congenita. The patient was asked to make a maximum voluntary contraction for three seconds, subsequently he was asked to relax the muscles of the hand as fast as possible. It took six seconds to totally relax the hand-muscles.

Pathophysiologically, NDMs belong to the skeletal muscle ion channelopathies. Skeletal muscle ion channels are voltage-gated ion channels that regulate the excitability of the skeletal muscle membrane (sarcolemma; see Clinical box 2). These ion channels are encoded by skeletal muscle ion channel genes. The skeletal muscle ion channelopathies are characterised by transient sarcolemma hyperexcitability (myotonia), hypoexcitability (paresis or paralysis) or both. This way of distinguishing the main symptoms corresponds well to the types of skeletal muscle ion channelopathies: 1. congenital myotonias without paralysis (chloride channelopathies) 2. congenital diseases with exclusively myotonia, exclusively paralysis or a combination of both (sodium channelopathies) and 3. congenital paralysis without myotonia (calcium channelopathies). The skeletal muscle ion channelopathies with their different types of symptoms and diseases are depicted in Figures 1.1a and 1.1b.

CLINICAL BOX 2

Muscle physiology

Striated muscles are organs specialised for a rapid generation of motion and force. They have the ability to shorten rapidly and efficiently, and to switch on and off in milliseconds. The activation of the contractile apparatus of striated muscles is the result of a cascade of events in response to an electrical signal received from the motor nerve: motor neuron activity transfers via the neuromuscular junctions to the sarcolemma, where action potentials propagate from the motor endplate zone along the muscle fibre towards each tendon (see Figure 1.3 for a schematic overview). Subsequently, the excitation spreads along the transverse tubular system into the depth of the muscle fibres, which is necessary for a contraction. This process is initiated by intracellular calcium release brought about by direct interaction of channel proteins of the transverse tubular system and the sarcoplasmic reticulum.

Inside the muscle cell, the electric potential is negative relative to its exterior. This so-called resting membrane potential, which is about -70 to -90 mV, sustained across the thin barrier of the sarcolemma, which has specific electrical properties for the maintenance of this potential. The basis of these properties lies in ion channels, which activate in response to ligands, transmitters, or voltage changes and inactivate by intrinsic inactivation processes. Voltage-gated ion channels are essential for the generation and modification of action potentials. The sarcolemma contains voltage-gated sodium, chloride, potassium and calcium channels. De- and repolarisations of the sarcolemma are regulated by fast moving ions that pass through these channels. With the membrane in rest, the Na gates of the Na channel are closed, the K gates of the K channels are closed and the Cl gates of the Cl channels are open. The normal resting membrane potential serves to keep the ion channels in an activatable state to accommodate for newly generated action potentials. An action potential is a transient reversal of the membrane potential caused by an alteration in the membrane's permeability. In the case of an action potential, Na channels open up, allowing Na^+ ions to enter the cell, causing a depolarisation. This action potential (with opening Na channels) is transmitted all along the sarcolemma. When the action potential has elapsed, the Na gates close and the K gates open, thus terminating the action potential. Shortly after, the K gates close and the Na-K pump in combination with the Cl channels will subsequently restore the resting membrane potential. In conclusion, a proper function of all these voltage gated ion channels is essential for a normal muscle contraction and relaxation.

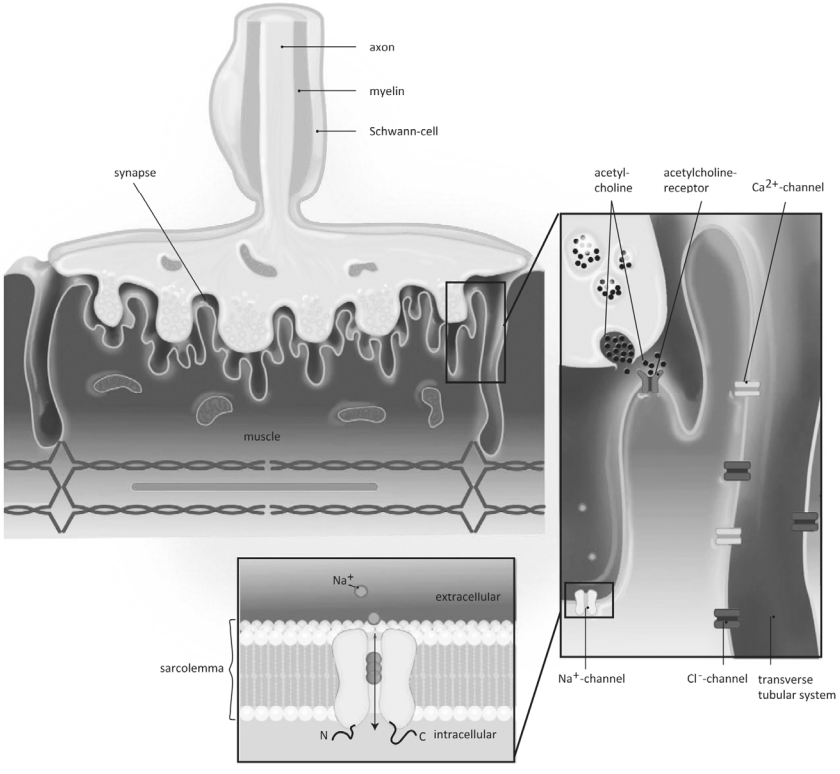

Figure 1.3 Schematic overview of the neuromuscular junction and the skeletal muscle fibre membrane (sarcolemma) with different ion channels. Enlarged is a sodium-channel during depolarisation. The action-potential is propagated along the sarcolemma. During depolarisation the sarcolemma is intracellular positively loaded and extracellular negatively loaded.

History of NDMs

Clinical features

Originally, a diagnosis of NDMs was based on clinical characteristics in combination with the hereditary pattern. In 1876 Thomsen, a Danish physician who suffered from the disease himself, was the first to describe the dominant form of myotonia congenita (DMC).[8] He chronicled the manifest stiffness (myotonia), which was most pronounced in the leg muscles, the reduction of stiffness through repetitive muscle contractions, which he coined the warm-up phenomenon, and dominant inheritance of the disease.[8] He also noted that the warm-up phenomenon was transient, diminishing after a few minutes of inactivity.

In 1957 Becker first described a recessive form of myotonia congenita (RMC) with a more generalised myotonia and the combination of myotonia and transient paresis.[9] Both symptoms improved with sustained exercise, which he also called the warm-up phenomenon.[5] The distribution pattern of RMC is reported as generalised: " [..] leg muscles are often severely affected and the muscles of the head, arms and hands are usually also involved".[10] As a rule, myotonia is first noticed in the legs,[11] which is why it is also called 'the ascending type of myotonia congenita'.[2] Since myotonia is defined as painless muscle stiffness, pain is reportedly uncommon.[10] The prevalence of RMC and DMC varies in different studies between 2 to 7.3 per 100,000.[3,12,13] The classical clinical picture of a patient with RMC is described in Clinical box 3.

CLINICAL BOX 3

Clinical picture of a patient with recessive myotonia congenita (M. Becker)
A 35-year-old road worker presented with a 30-year history of skeletal muscle stiffness. He occasionally experienced fell when attempting to rise from a chair or walk or run rapidly. He described his symptoms as follows: "It is often impossible for me to get up from a chair. When I get up quickly, my leg muscles seize up and remain contracted (consistent with myotonia) followed by a brief sensation of weakness (compatible with transient paresis). Every attempt at moving forward is difficult and I even fall at times. When I contract my muscles several times in succession, they become less stiff and strength returns (analogous to the warm-up phenomenon). After a few minutes I will be as strong and flexible as other people." He continued: "At work I have to keep moving all the time, especially before crossing the street, to prevent myself from falling or to make sure it won't take me longer to cross the street than planned." The leg stiffness had become more pronounced over the years, with stiffness in the hands having first become manifest 15 years ago. There was no family history of any similar disorder and the clinical and electromyographic evaluations of his parents were completely normal.

Clinical examination of the patient revealed pronounced muscular hypertrophy, especially in the legs, thighs, and shoulders. There was no eyelid myotonia, but a marked handgrip myotonia (action myotonia). On arising from a chair the patients gait was stiff-legged with a minor weakness lasting approximately 10 to 15 seconds. Percussion myotonia was easily elicited from the thenar and large muscles of the legs. The other results of the physical examination were all normal.

Needle-electromyography (EMG) showed continuous myotonic discharges in almost all skeletal muscles examined. Figure 1.4 shows a myotonic discharge in the patient's left biceps. Direct nucleotide sequence analysis yielded two different mutations: c.501C>G; p.F167L and c.1238T>G; p.F413C in the *CLCN1*-gene (compound heterozygous). The patient's father appeared to be a carrier of the F413C and his mother a carrier of the F167L mutation.

Treatment with mexiletine 3 dd 200 mg diminished the myotonic symptoms.

Paramyotonia congenita (PC) was first described by Von Eulenburg in 1886.[6] This dominant disease differs from myotonia congenita in various aspects. First, the myotonia worsens after repetitive contractions (paramyotonia). Second, it tends to be provoked and exacerbated by low temperatures.[6] Third, patients with PC may show muscle weakness provoked by prolonged exercise or low temperatures. Contrary to

transient paresis, muscle weakness in PC may persist for hours. In general, muscles are bilaterally and symmetrically affected.[10] In his original report, Eulenburg described a predilection of myotonia for facial, tongue, throat and hand muscles.[6] Streib later confirmed this.[14] Also in PC pain is uncommon.[10] The prevalence of PC has been estimated at 1 per 356,000.[15]

Hyperkalemic periodic paralysis (hyperPP) is a dominant condition, which formally belongs to the periodic paralyses. It was first described in the 1950s.[16,17] However, it forms a continuum with PC: patients with hyperPP may show some paramyotonia while patients with PC may be accompanied by sequences of minor attacks of periodic paralysis. HyperPP is mainly characterised by episodes of generalised muscle weakness. Often, but not always, an increase in serum potassium can be established during an attack of generalised muscle weakness. Potassium-rich meals, a period of rest after exercise, emotions/stress, pregnancy and cold environment tend to trigger or exacerbate the attacks.[18] The condition has a prevalence of approximately 1 in 200,000.[10]

Pathophysiology of and genes implicated in NDMs

In 1969 Bryant was the first to provide initial insight into the pathophysiological mechanism underlying myotonia by showing that the membrane resistance of myotonic goat muscle fibres was considerably elevated at rest, which he later found to be due to a strongly diminished sarcolemmal chloride conductance.[19,20] Two decades later Rüdel confirmed this diminished sarcolemmal chloride conductance in a mouse model.[21] The same phenomenon was also shown to underlie DMC and RMC in humans.[22-25] DMC and RMC are thus caused by a permanent reduction of the resting chloride conductance of the sarcolemma.[23,24] A normal chloride conductance is necessary for a fast repolarisation of the sarcolemma and a strongly reduced chloride conductance will result in a lack of functional chloride channels, which, in turn, results in a higher resting membrane potential rendering the sarcolemma vulnerable to depolarisations.[26] A 30% reduction in chloride conductance may cause the overexcited sarcolemma to produce repetitive depolarisations (myotonia).[27] When the resting membrane potential is much higher, the sarcolemma may temporarily become inexcitable, forming the basis for transient paresis.

In the 1980s *in vitro* electrophysiological studies with skeletal muscle fibres of PC patients uncovered a disturbed fast inactivation (impaired repolarisation) of sodium channels.[28-30] Accordingly, PC is caused by a long-lasting depolarisation of the sarcolemma due to an inactivation defect of the sodium channel.[29,31] When the preceding depolarisation is limited, the already recovered sodium channels can be reactivated immediately after their repolarisation, which leads to an overexcited sarcolemma with repetitive depolarisations (myotonia).[26] However, when the

preceding depolarisation is strong, the inactivated state of the sodium channels is prolonged, leading to an inexcitable sarcolemma resulting in periodic paralysis.[27]

In the early 1990s genetic studies of both forms of myotonia congenita were linked to the skeletal muscle chloride channel gene (*CLCN1*), mapped to chromosome 7q35. This gene encodes the chloride channel (ClC-1;voltage-gated chloride channel, type 1) in the sarcolemma (Figure 1.3).[32-34] Subsequent molecular genetic studies demonstrated the first point mutations in this gene.[32,35-37] So far, approximately 80 different mutations have been associated with myotonia congenita.[38,39]

In the same period, different research teams independently linked PC and HyperPP to the *SCN4A* gene, which was genetically mapped to chromosome 17q23-25.[40-43] This gene encodes the pore-forming α-subunit (hSkM1) of the skeletal muscle sodium channel (NaV1.4; voltage-gated sodium channel, type 4A) in the sarcolemma (Figure 1.3).[44] By identifying different point mutations in *SCN4A* it was established that this was the disease causing gene.[45-48] Yet, at least 30 different missense mutations underlying these three groups of sodium channel disorders have meanwhile been identified.[49,50]

Reclassification of NDMs

Following the advances in DNA-analysis a third group of disorders associated with myotonia and caused by a disturbed, fast inactivation of sodium channels was uncovered that was most likely formerly diagnosed as DMC, i.e., potassium-aggravated myotonias (PAM).[7,51-53] PAM is a collective term for unusual myotonias resulting from potassium sensitivity, i.e., myotonia fluctuans (fluctuating myotonia), myotonia permanens (permanent myotonia), and acetazolamide responsive myotonia congenita.[7,54] Typical of myotonia fluctuans is its erratic muscle stiffness.[7] Another hallmark of this condition is the so-called “exercise-induced delayed onset myotonia”, in which the muscle stiffness only appears after prolonged exercise. Myotonia permanens is characterised by a serious and continuous myotonia, which may clinically be difficult to differentiate from myotonia congenita. Finally acetazolamide-responsive myotonia congenita is a painful PAM that responds well to acetazolamide.[54] There are several other PAMs that have also been associated with painful myotonia.[2,55,56] None of the PAMs involve cold sensitivity or muscle weakness. Rüdel et al. suggested to refere to PAM diagnosed without a potassium-loading test as a sodium-channel myotonia (SCM), [57] which term we will use in this thesis.

Over the past decade, combined, the pathophysiological and genetic studies described above have led to a reclassification of the NDMs.[58-60] NDMs are now categorised as two important groups consisting of chloride and sodium

channelopathies (Figure 1.5). Chloride channelopathies (ClCh) comprise autosomal-recessive (RMC; M. Becker) and autosomal-dominant myotonia congenita (DMC; M. Thomsen), while sodium channelopathies (NaCh) include paramyotonia congenita (PC), potassium-aggravated myotonias (PAM) and hyperkalemic periodic paralysis with (para)myotonia (HYPP).

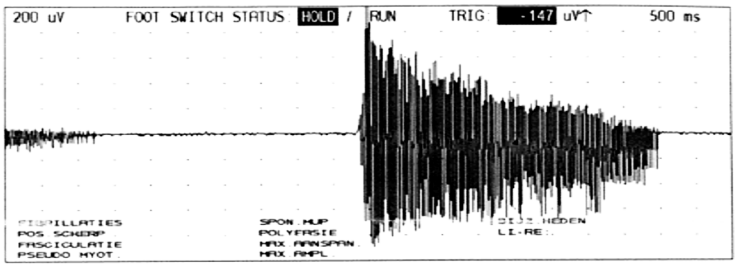

Figure 1.4 Concentric needle-EMG of the left biceps brachii, showing a myotonic discharge.

Ancillary investigations

Needle EMG almost invariably show myotonic discharges in patients with a NDM, which audible discharges are especially triggered by insertion or manipulation of the needle and typically wax and wane in frequency and amplitude (Figure 1.4). The characteristic sound, that of an accelerating motorcycle, is caused by the changes in frequency.[61] These myotonic discharges are detectable in almost all skeletal muscles.[62,63] Analysing electrical and mechanical phenomena of myotonia in patients with myotonia congenita, Wagner found a correlation between myotonic muscle fibre discharges and mechanical findings depending on the frequency of stimulation and the force of contraction.[62] Comparing ClCh and NaCh, Fournier detected no differences in the quantity of myotonic discharges.[63]

Transient paresis can be indirectly confirmed by means of neurophysiological tests.[5,64-66] A strong correlation has been reported for transient paresis and the overall decrement of the compound muscle action potential (CMAP) after repetitive nerve stimulation (RNS).[64,65] However, a certain decrement can be found in all types of myotonia.[66] On the other hand, some NDM subtypes may be distinguished by the differences in CMAP output after short or long exercise tests.[67,68] Fournier described three EMG patterns, each correlating with a subgroup of mutations.[69] Furthermore, transient paresis can be measured and explained using force measurements and high-density surface EMG.[5]

Lastly, although DNA analysis for NDMs became available in the 1990s, procedures may be complex and complicated. In some countries screening for *CLCN1* or *SCN4A*

mutations may take up to an entire year. Moreover, 25 to 60% of the DMC and RMC patients lacked any identifiable *CLCN1* mutation.[70-72] To date, comparable studies in patients with PC, PAM or HYPP have not yet been performed.

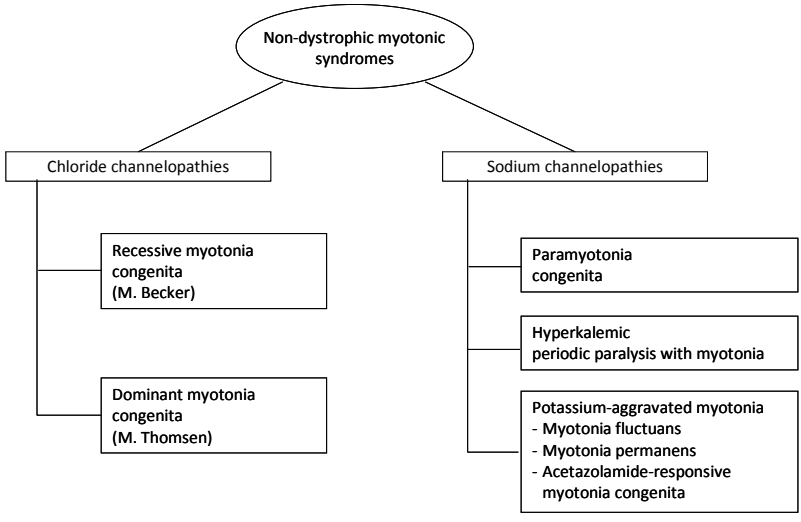

Figure 1.5 Non-dystropic myotonic syndromes devided in chloride and sodium channelopathies.

Differential diagnoses

Myotonia

The principal differential diagnosis of myotonia consists of the dystrophic myotonic syndromes like myotonic dystrophy type 1 (DM1) and type 2 (DM2). DM1 is more frequent than NDMs and DM2. Furthermore, DM1 is an autosomal, dominant disease in which the pathology is not only limited to the sarcolemma itself. It is a multi-system disorder, which is accompanied by a characteristic distribution of progressive muscle weakness (initially in the muscles of the face, throat, neck, and distal limbs, with severer symptoms developing in the proximal limb muscles in a later stage of the disease), and the involvement of different organs, i.e., the heart (conduction defects and arrhythmias), the eyes (cataract), smooth muscles (gastro- intestinal complaints), lungs (aspiration pneumonia), and the brain (mental retardation and hyper-somnolence).[73]

DM2, also denoted as proximal myotonic myopathy (PROMM), is a disease mimicking DM1.[74] The main distinction between DM1 and DM2 is that patients with DM2 show

pronounced proximal muscle weakness in combination with myalgia. Contrary to DM1 there is hardly any cognitive impairment, hypersomnia, ptosis, muscle wasting or respiratory insufficiency. The two disorders can be distinguished via DNA analysis.

Periodic paralyses

It is important to differentiate hypokalemic periodic paralysis and thyrotoxic periodic paralysis from hyperkalemic periodic paralysis. Hypokalemic periodic paralysis mimics the hyperkalemic variant, except that during a generalised attack of muscle weakness a decreased serum potassium is measured. Moreover, the syndrome will never show myotonia or myotonic discharges. Like the other two diseases, thyrotoxic periodic paralysis is characterised by identical attacks of generalised muscle weakness and there may also be a low serum potassium. At the time of the first attack the clinical thyrotoxic symptoms may go undetected.[10] Thus, in cases of periodic paralyses, especially in hypokalemic periodic paralysis, it is recommended to always check the patient's thyroid function.

Treatment

Treatment of NDMs is, to date, purely symptomatic. Myotonia may respond to drugs reducing or blocking the sodium channels of the sarcolemma.[75-77] These agents alleviate myotonia by reducing the hyperexcitability of this membrane in both ClCh and NaCh. The first pharmacological treatment for myotonia was published by Wolf in 1936 who treated four patients with myotonia congenita with quinine, an anti-arrhythmic drug.[78] Other authors later reported favourable effects for procainamide, tocainide, and phenytoin.[75,76,79-82] Nevertheless, expert opinion suggests mexiletine as the drug of choice.[10,12] However, the effects of mexiletine in patients with myotonia has so far only been tested in some case reports,[83-85] one study with a heterogeneous population,[75] and one with an electrophysiological evaluation.[86] Finally, the usefulness of acetazolamide, a carbonic anhydrase inhibitor, in the treatment of myotonia has been described for some sodium channelopathies.[87,88] In conclusion it needs to be pointed out that none of the mentioned regimes is an established or evidence-based treatment for myotonia.

Thesis rationale

To summarise, NDMs were considered a separate clinical entity for more than a century. In the pre-genetic era, clinical features were already described thoroughly, most particularly by Thomsen, Eulenburg and Becker.[3,6,8] In the seventies and eighties of the previous century researchers detected chloride conductance disturbances in the sarcolemma of myotonic goats. In *vitro* electrophysiological studies with skeletal muscle fibres of patients diagnosed with PC, moreover, uncovered sodium conductance disturbances. Subsequently, the responsible genes, *CLCN1* and *SCN4A*, were identified. Since then, scientific research in this field has been chiefly dedicated to the molecular and genetic pathophysiology of the syndromes. Subsequently, several independent authors reported genotype-phenotype mismatches.[56,57,89,90] The warm-up phenomenon had, for instance, always been assumed to be a typical symptom for chloride channelopathies. However, instead of an expected chloride channel defect, several researchers detected a mutation in the skeletal muscle sodium channel gene in patients and families displaying this phenomenon.[56,89,90] It hence became apparent that existing, assumed genotype-phenotype correlations were imprecise and that the clinical pictures of the various NDM subtypes as presented in the literature could be inaccurate. Obviously, the genotype is necessary to redefine the pure clinical phenotypes of NDMs, [2,57,89,91-93] which has now become feasible thanks to the current DNA analysis technology that allows us to compose homogeneous groups of patients characterised either by chloride or sodium channel abnormalities. However, to date, the literature on phenotypic characteristics of a group of NDM patients with genetically confirmed ClCh and NaCh is scarce. Recently, Fialho et al. were the first to report on a large cohort with genetically confirmed ClCh, while Matthews and colleagues described a cohort of genetically confirmed NaCh cases.[94,95]

Building on these preliminary findings, the aim of the present thesis is to describe the phenotypes of NDMs on basis of their genetics, to provide clinicians with rules of thumb for focussed genetic testing, and to create a better strategy to detect genetic mutations. Lastly, we would like to create better circumstances for the development of double-blind randomised clinical trials (RCTs).

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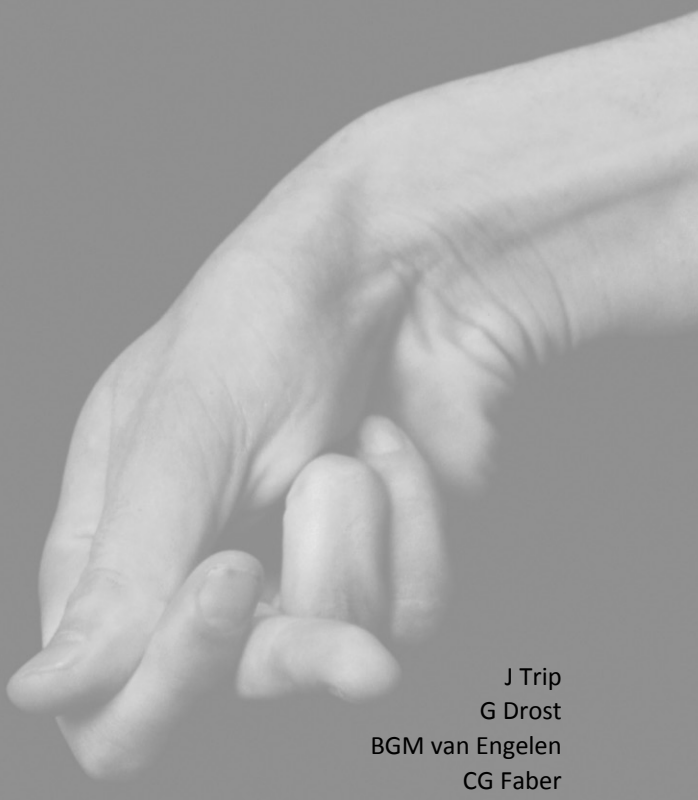
Chapter 2

Drug treatment for myotonia: a systematic review



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Synopsis

Drug therapy to treat myotonia (delayed muscle relaxation after contraction) in muscle diseases such as myotonic dystrophy and myotonia congenita.

Myotonia is an abnormal relaxation of muscles after contraction. It is a key symptom in a number of muscle diseases called myotonic disorders. It can be mild or severe, interfering with daily activities such as walking, climbing stairs or opening and closing the eyelids. It can be worse after periods of rest or triggered by cold or fatigue. People with mild myotonia can manage their disease without medication but in severe cases treatment is usually necessary. Drugs that have been used to treat myotonia include sodium channel blockers such as procainamide, phenytoin and mexiletine; tricyclic antidepressant drugs such as clomipramine or imipramine; benzodiazepines; calcium antagonists; taurine and prednisone. This review describes ten randomised controlled trials which tested the effectiveness of twelve different drug treatments. The ten trials included a total of 143 patients of which 113 had myotonic dystrophy and 30 had myotonia congenita. The trials were generally small and of poor quality. Meta-analysis was not possible due to a lack of appropriate trials and data. Two small studies indicated that clomipramine and imipramine had a short-term beneficial effect on the myotonia in myotonic dystrophy and one small study indicated that taurine had a long-term beneficial effect on the myotonia in myotonic dystrophy. Minor side effects such as dry mouth and dizziness were reported with clomipramine and imipramine, but not with taurine. Based on the evidence from the ten trials included in this review, it was not possible to determine whether drug treatment is safe and effective for myotonia in people with a myotonic disorder. Larger, well-designed randomised controlled trials are needed.

Abstract

Background

Abnormal relaxation of skeletal muscles, known as myotonia, can cause disability in myotonic disorders. Sodium channel blockers, tricyclic antidepressive drugs, benzodiazepines, calcium-antagonists, taurine and prednisone may be of use in reducing myotonia.

Objectives

To consider the evidence from randomised controlled trials on the efficacy and tolerability of drug treatment in patients with clinical myotonia due to a myotonic disorder.

Search strategy

We searched the Cochrane Neuromuscular Disease Group trials register (July 2009), MEDLINE (January 1966 to July 2009) and EMBASE (January 1980 to July 2009). Grey literature was hand-searched and reference lists of identified studies and reviews were examined. Authors, disease experts and manufacturers of anti-myotonic drugs were contacted.

Selection criteria

We considered all (including quasi) randomised trials of participants with myotonia treated with any drug treatment versus no therapy, placebo or any other active drug treatment.

The primary outcome measure was reduced clinical myotonia using two categories: (1) no residual myotonia or improvement of myotonia or (2) no change or worsening of myotonia.

Secondary outcome measures were: (1) clinical relaxation time; (2) electromyographic relaxation time; (3) stair test; (4) presence of percussion myotonia; and (5) adverse events.

Data collection & analysis

Two authors extracted the data independently onto standardised extraction forms and disagreements were resolved by discussion.

Main results

Ten randomised controlled trials were found comparing active drug treatment versus placebo or another active drug treatment in patients with myotonia due to a myotonic disorder. Included trials were double-blind or single-blind crossover studies involving a total of 143 patients of whom 113 had myotonic dystrophy type 1 and 30 had myotonia congenita. The studies were of poor quality. Therefore, we were not able to analyse the results of all identified studies. Two small crossover studies without a washout period demonstrated a significant effect of imipramine and taurine in myotonic dystrophy. One small crossover study with a washout period demonstrated a significant effect of clomipramine in myotonic dystrophy. Meta-analysis was not possible.

Reviewers' conclusions

Due to insufficient good quality data and lack of randomised studies, it is impossible to determine whether drug treatment is safe and effective in the treatment of myotonia. Small single studies give an indication that clomipramine and imipramine have a short-term beneficial effect and that taurine has a long-term beneficial effect on myotonia in myotonic dystrophy type 1. Larger, well-designed randomised controlled trials are needed to assess the efficacy and tolerability of drug treatment for myotonia.

Background

Myotonia is a clinical phenomenon, which refers to an abnormal muscle relaxation after voluntary or evoked muscle contraction.[1] It is a cardinal feature in myotonic disorders including the dystrophic myotonic syndromes and the non-dystrophic myotonic syndromes. Myotonia may be present in every skeletal muscle. Clinical examination reveals action myotonia and/or percussion myotonia both of which are best tested in the hand muscles: following a forceful grip, the ability to relax the grip is delayed (action myotonia or grip myotonia); or mechanical stimulation, for example a blow with the percussion hammer on the thenar muscles, will also contract the muscle for a few seconds (percussion myotonia). Furthermore, an acute muscle contraction may give a transient decline in muscle force (transient paresis).[2,3] Repeated muscle contraction and relaxation may improve myotonia as well as muscle force, which is called the 'warm-up' phenomenon. However, in a condition called paramyotonia, the myotonia worsens after repetitive contractions (paradoxical myotonia).

A number of conditions are associated with an abnormal relaxation of muscles in a way that resembles myotonia but they do not have the characteristic electrophysiological features of true myotonia (pseudomyotonia).[4] Because such pseudomyotonia may have a different physiological basis from true myotonia, we excluded these conditions from our review. These conditions include McArdle's disease (Glycogenosis type V), Hoffman's disease (myotonia in hypothyroidism), Brody's disease (sarcoplasmic reticulum-Ca2+ATPase deficiency), neuromyotonia, neuroleptic malignant syndromes and tetanus. Schwartz-Jampel syndrome (chondrodystrophia myotonia) was also excluded because myotonic activity in this disease persists during general anesthesia, which does not happen in true myotonia.[5] The true myotonic syndromes included in this review are discussed below.

Dystrophic myotonic syndromes

Myotonic dystrophy type 1 is an autosomal-dominant disorder in which myotonia is accompanied by a characteristic pattern of muscle weakness and by the involvement of several organs.[6-8] This condition is caused by an expanded CTG (cytosine-thymine-guanine) trinucleotide repeat in the DMPK-gene on chromosome 19q.[9,10] The inheritance is characterised by anticipation, that is the earlier and more severe onset of the disease in successive generations.[11] The prevalence of myotonic dystrophy type 1 varies from 2 to 12 per 100,000.[12] Myotonia is clinically detectable in almost every symptomatic patient. Recently, myotonic dystrophy type 2 was described, which differs from type 1 in its predominant proximal muscle weakness. It was, therefore, originally named proximal myotonic myopathy (PROMM).[13,14] Myotonic dystrophy type 2 is caused by an increased CCTG repeat in the ZNF9 gene on chromosome 3. We

have included patients with clinical myotonia due to both types of myotonic dystrophy.

Non-dystrophic myotonic syndromes

Clinically non-dystrophic myotonic syndromes have myotonia with or without periodic paralysis.[15] Recently the molecular basis of these disorders has been discovered. No obvious genotype-phenotype correlations exist.[16-18] Over the past decade, a combination of electrophysiological and molecular biological studies have led to a reclassification of this group of diseases.[2,15,19,20] They are now classified as chloride (ClCh) or sodium channelopathies (NaCh).

There are two forms of ClCh: autosomal-recessive myotonia congenita (RMC; M. Becker)[21,22] and autosomal-dominant myotonia congenita (DMC; M. Thomsen).[23] Both diseases are characterised by clinical myotonia. Autosomal-recessive myotonia congenita also shows transient paresis.[2,3] The disorders are caused by a mutation in the skeletal muscle chloride channel gene (*CLCN1*) on chromosome 7q.[24-26] The prevalence of ClCh varies in different studies between 2 to 7.3 per 100,000.[22,27,28] We included all patients with dominant and recessive myotonia congenita in our review.

NaCh are all autosomal-dominantly inherited or sporadic and are divided into paramyotonia congenita (PC), potassium-aggravated myotonia (myotonia fluctuans, myotonia permanens and acetazolamide responsive myotonia congenita) and hyperkalemic periodic paralysis (hyperPP).[29-33] The NaCh are caused by a mutation in the skeletal muscle sodium channel gene (*SCN4A*) on chromosome 17q encoding for SkM1, the alpha-subunit of the sodium channel.[24,34] The exact prevalence of sodium channel diseases is not known although the prevalence of PC has been estimated at 1 per 356,000.[21] HyperPP can occur with myotonia or paramyotonia and sometimes without either. We excluded HyperPP without (para)myotonia and included all other sodium channel disorders in our review.

The pathophysiological mechanisms in the several myotonic disorders are different. Recent publications suggest that the expanded CTG-repeat in myotonic dystrophy triggers aberrant splicing of chloride channel mRNA but it is also possible that the myocytes in myotonic dystrophy display an abnormal Na+ channel activity.[35-37] Thus, the exact pathophysiological mechanism leading to myotonia in myotonic dystrophy is unknown.

The ClCh are caused by a permanent reduction of the resting chloride conductance of the sarcolemma.[38,39] Normal chloride conductance is necessary for a fast repolarisation of the sarcolemma, otherwise the sarcolemma tends to stay

depolarised causing myotonia or becomes hyper-depolarised causing a loss of excitability and thereby a transient paresis.[40]

NaCh are caused by a long-lasting depolarisation of the sarcolemma due to an inactivation defect of the sodium channels.[41,42] These can initiate successive action potentials, which is the basis for myotonia.[40]

Many people with mild myotonia can manage their disease without medication. Severe myotonia can interfere with daily activities and in these individuals treatment is often necessary. No treatment for the cause of myotonia is available, so treatment is merely symptomatic. In general drugs that block the sodium channels, independent of the disease process involved, may diminish myotonia. These agents reduce the excitability of the cell membrane of the skeletal muscle and include local anesthetics and cardiac agents such as anti-arrhythmic drugs.

The first treatment for myotonia was published by Wolf in 1936 who treated four patients with myotonia congenita with quinine, an anti-arrhythmic drug.[43] The literature also suggests that procainamide, tocainide and phenytoin have favourable effects.[44-49] However, procainamide and tocainide could have serious long-term side effects. Expert opinion suggests that mexiletine is the agent of first choice.[28] However, the published evidence basis for this opinion is unclear. There are some case reports, one study with a heterogeneous population and an electrophysiological evaluation on the use of mexiletine in patients with myotonia.[45,50-53] Acetazolamide is a carbonic anhydrase inhibitor traditionally thought of as a diuretic, but it has been described as useful for myotonia in some NaCh.[54,55]

A crucial aspect to this review is how to quantify myotonia because it can be difficult to standardise this as highlighted by a report of an experimental protocol to quantify myotonia.[56] The problems include the variability of the myotonia between patients and within a given patient at different times of the day, and how to take account of the warm-up phenomenon. Furthermore, there is the usual problem of inter-rater variability. A possible solution for the last item might be the use of specific devices with a computerised protocol.[1,57] At the moment, one of the most used parameters of myotonia is the relaxation time after maximum voluntary contraction (MVC) as measured by stopwatch, special technical equipment or computerised protocols. A related measure is the electromyographic variant, the electromyographic (EMG) relaxation time after MVC. Another used parameter is to record the presence or absence of percussion myotonia. These parameters measure the impairment, but not the functional effect of myotonia. The stair test (time needed to climb ten stairs) is possibly the best available method for measuring approximate functional benefit.

No systematic reviews of drug treatment for myotonia are known. Two non-systematic reviews of therapy for the myotonic disorders have been published.[58,59] This systematic review aims to provide the evidence on which to base treatment.

Objectives

To consider the evidence from randomised controlled trials on the efficacy and tolerability of drug treatment in people with clinical myotonia due to a myotonic disorder.

Criteria for considering studies for this review

Types of studies

We included all randomised and quasi-randomised (alternate or other systematic treatment allocation) trials of any drug treatment in people with clinical myotonia due to one of the myotonic disorders described below.

Types of participants

Participants of all ages with clinical myotonia caused by myotonic disorders, such as the dystrophic myotonic syndromes and the non-dystrophic myotonic syndromes, were included. It is now possible to diagnose the myotonic disorders by DNA-analysis. This was not possible at the time when the included studies were performed, so DNA-analysis was not an inclusion criterion in our review.
We excluded people with McArdle's disease (Glycogenosis type V), Hoffman's disease (myotonia in hypothyroidism), Brody's disease (sarcoplasmic reticulum-Ca2+ATPase deficiency), neuromyotonic diseases, neuroleptic malignant syndromes, tetanus and Schwartz-Jampel syndrome. For trials or treatment groups including people with dystrophic myotonic syndromes and non-dystrophic myotonic syndromes we described the different diseases and the degree of myotonia separately, if this was possible.

Types of interventions

We included any drug treatment (given either singly or in combination) versus no therapy, placebo or another active drug treatment. The list of potential drugs included quinine, procainamide, tocainide, phenytoin, mexiletine and acetazolamide, but this list was not exclusive.

Types of outcome measures

Primary outcome

As there is no consensus regarding the best measure of myotonia leading to disparate outcome measures in each of the randomised controlled trials, we devised a measure using a categorisation of the changes in clinical myotonia after drug treatment for each trial based on the conclusion of the original authors as follows:
(1) improvement of myotonia with no residual clinical myotonia;
(2) improvement of myotonia but still clinically detectable;
(3) no change of myotonia;
(4) worsening of myotonia.

Secondary outcomes

(1) Relaxation time: the time taken to fully open the hand after a maximum voluntary contraction (MVC) (hand-grip myotonia). This might be determined manually by stopwatch or by computerised protocols. When using a computerised hand-grip myometer the decline in maximum voluntary contraction from 90 to 5% during relaxation is frequently used to measure the relaxation time. However, some researchers have used 50%, 75% or 100% decline from peak MVC as the relaxation time. We included all such protocols.
(2) Electromyographic (EMG) relaxation time: the phenomenon of myotonia can be recorded with an electromyographic needle electrode and is seen as positive waves, so called myotonic discharges or after-discharges. After MVC these myotonic or afterdischarges wax and wane and finally stop. The duration of these after-discharges is also called EMG relaxation time. For example after-discharges can be recorded from the opponens pollicis muscle.
(3) Stair test: time needed to climb ten stairs.
(4) Presence of percussion myotonia: percussion myotonia is myotonia occurring after a mechanical stimulus; for example tested using percussion of the thenar muscles of the hand with a reflex hammer.
(5) The occurrence of one or more adverse events during treatment with the different agents. We specified the adverse events.
For all outcome measures we used a minimum treatment duration of one week and a maximum treatment duration of one year and where necessary planned to adjust for different follow-up periods.

Search strategy for identification of studies

See: Cochrane Neuromuscular Disease Group search strategy.
The Cochrane Neuromuscular Disease Group trials register was searched for randomised controlled trials using: "myotonia", "myotonic dystrophy"', "non-dystrophic myotonias", "myotonia congenita", "Morbus Thomsen", "Morbus Becker",

"potassium-aggravated myotonia", "myotonia fluctuans", "myotonia permanens", "paramyotonia congenita", "hyperkalemic periodic paralyses", "relaxation" AND "muscle" and "treatment" OR "therapy" as the primary search items (July 2009). We adapted this strategy to search MEDLINE (January 1966 to July 2009) and EMBASE (January 1980 to July 2009) for other randomised controlled trials. Grey literature such as neuromuscular text books and abstracts from international neuromuscular congresses were hand-searched and we checked the reference lists of the identified literature and reviews concerning myotonia. We also contacted authors, disease experts and manufacturers of anti-myotonic drugs.

Electronic search strategies

EMBASE strategy

((randomised controlled trial or clinical trial or multicenter study or controlled study or crossover procedure or double blind procedure or single blind procedure or randomisation or major clinical study or placebo or meta analysis) or (phase 2 clinical trial or phase 3 clinical trial or phase 4 clinical trial) or (clin* and trial*) or ((singl* or doubl* or tripl* or trebl*) and (blind* or mask*)) or placebo* or random* or control* or (meta?analys* or systematic review*) or (cross?over or factorial or sham? or dummy) or ABAB design*) not (animal)
AND
((myotonia OR myotonic dystrophy OR myotonic disorders OR non-dystrophic myotonia OR myotonia congenita OR morbus Thomsen OR morbus Becker OR potassium aggravated myotonia OR myotonia fluctuans OR myotonia permanens OR paramyotonia congenita OR hyperkalemic periodic parlalysis) OR (muscle AND relaxation) AND (treatment OR therapy))

MEDLINE strategy

(randomised controlled trial [PT] OR randomised controlled trials OR controlled clinical trial [PT] OR controlled clinical trials OR random allocation OR double-blind method OR single-blind method OR clinical trial [PT] OR exp clinical trials OR (clin* AND trial*) OR ((singl* OR doubl* OR trebl* OR tripl*) AND (blind* OR mask* OR dummy)) OR placebos OR placebo* OR random* OR research design OR (clinical trial phase I OR clinical trial phase ii OR clinical trial phase iii OR clinical trial phase iv [PT]) OR multicenter study [PT] OR meta analysis [PT] OR prospective studies OR intervention studies OR cross-over studies OR meta-analysis OR (meta?analys* OR systematic review*) OR control*) NOT (Animal [MESH] NOT (Human [MESH] AND Animal [MESH])) AND ((myotonia OR myotonic dystrophy OR myotonic disorders OR non-dystrophic myotonia OR myotonia congenita OR morbus Thomsen OR morbus

Becker OR potassium aggravated myotonia OR myotonia fluctuans OR myotonia permanens OR paramyotonia congenita OR hyperkalemic periodic paralysis) OR (muscle AND relaxation) AND (treatment OR therapy))

Methods of the review

Selecting trials for inclusion

Two authors (JT and CGF) independently reviewed the titles and abstracts from the electronic search to identify relevant trials for full review. The full text of all potentially relevant studies was obtained for assessment. The authors decided which trials fitted the inclusion criteria and graded their methodological quality. Disagreement was resolved by discussion. Review authors were not blinded to trial authors' names, institutions and the journals of publication.

Assessment of methodological quality

Two independent authors (JT and CGF) assessed randomised trials for methodological quality with respect to the following items: allocation concealment, patient blinding, observer blinding, explicit diagnostic inclusion and exclusion criteria and explicit outcome measures. These items were assessed according to the Cochrane approach: A - adequate, B - unclear, C - inadequate, D - not done. Disagreement was resolved by discussion.

Data extraction

Data extraction on participants, methods, intervention, outcomes and adverse events was performed by two independent authors (JT and CGF) using a data extraction form. We attempted to obtain missing data from the trial authors if this was necessary. For the primary outcome we had created a special scoring system: (1) no residual clinical myotonia; (2) improvement of myotonia but still clinically detectable; (3) no change; (4) worsening of myotonia. Data were transformed from the original studies by two authors (JT and CGF) with any disagreement being resolved by discussion.

Analysis

For statistical analysis of the primary outcome we dichotomised the variable scoring system:
(1) no residual myotonia or an improvement;
(2) no change or worsened.

Relative risks (RR) with 95% confidence intervals (CI) were calculated from the dichotomised data for each study if this was possible. Where possible the numbers needed to treat (NNT) and the numbers needed to harm (NNH) would also have been calculated. If all necessary data could be deduced from the published results, the primary outcome for crossover studies were analysed using the McNemar's test, calculating the odds ratios.[60,61] If there had been continuous data in the secondary outcomes we would have calculated the weighted mean difference (WMD) with 95% CI or presented the original statistical analysis of the study. If there had been more than one trial with the same agent in the same disease group we would have calculated a weighted treatment effect across those trials using a fixed-effect model with the Cochrane statistical package, Review Manager (RevMan). We interpreted a p-value less than or equal to 0.05 as statistically significant. If chi-squared analysis showed heterogeneity of the study results (p-value<0.1), sensitivity analyses would have been carried out to explore plausible causes. If heterogeneity could still not be explained, we would have reported the results using a random-effects model. We would have analysed the dystrophic myotonic syndromes and the non-dystrophic myotonic syndromes as subgroups if possible. We also discussed adverse events and cost benefits drawing upon non-randomised data.[62]

Description of studies

See Table 2.1 characteristics of included studies and Table 2.2 characteristics of excluded studies.

Table 2.1 Characteristics of included studies

Study	Antonini 1990[63]
Methods	Randomised, double blind crossover study. Method of randomisation not stated. Single centre Italy. Treatment period of 33 days, total duration 166 days. Results presented as combined data from both active treatment arms and both placebo arms. Two washout periods of 30 days. Results first arms stated.
Participants	17 patients with 2 withdrawals. 17 patients with myotonic dystrophy. 8 patients were male, 9 female. Mean age 29 (SD not stated). Inclusion criteria: well-established criteria for myotonic dystrophy. Exclusion criteria: Subjects with cardiac, ophthalmologic, or urologic diseases.
Interventions	Clomipramine 75 mg/day. Comparison treatment placebo.
Outcomes	Grip myotonia by relaxation time in seconds; time necessary to completely open the fist after three seconds of maximum voluntary contraction performed by maintaining a constant pressure in a rolled sphygmomanometer cuff.
Allocation concealment	B
Study	Durelli 1983[64]
Methods	Randomised, double-blind crossover study. Methods of randomisation not stated. Single centre Italy. Treatment periods of 6 months, total duration of 1 year. Results presented as combined data from both active treatment arms and both placebo arms. No washout period. Results first arms not stated.

Characteristics of included studies (Continued)	
Participants	9 patients without withdrawals. 9 patients with myotonic dystrophy. Number of males and females not stated. Mean age not stated. Inclusion criteria: established clinical EMG-criteria Exclusion criteria: None stated
Interventions	Taurine 100-150 mg/kg. Comparison treatment placebo.
Outcomes	EMG relaxation time after maximum voluntary contraction, occurrence of percussion myotonia, occurrence of myotonic discharges by electrical stimulation of median nerve, KCl loading test in mmol/litre necessary for occurrence of myotonia.
Allocation concealment	B
Study	Finlay 1982 [69]
Methods	Randomised, double-blind crossover study. Method of randomisation not stated. Single centre United Kingdom. Treatment periods of 14 days, total duration 28 days. Results presented as descriptive, individually data for the four treatment arms. No washout period. Descriptive data first arms stated.
Participants	10 patients with 2 withdrawals. 10 patients with myotonic dystrophy. 7 patients were male, 3 female. Mean age not stated. Range from 31-59 years. Inclusion criteria: none stated. Exclusion criteria: none stated.
Interventions	Procainamide 250 mg 4x/day first week and 500 mg 4x/day second week versus disopyramide 100 mg 3x/day first week and 200 mg 3x/day second week. Comparison between both treatments.
Outcomes	Grip myotonia by measuring relaxation time in seconds necessary to completely open the fist after three minutes of maximum voluntary contraction, grip strength by using RAF Gripometer, subjective comments.
Notes	Individually continuous data not stated, no statistical analysis, patients could be recognise their original medicine by kind of adverse events.
Allocation concealment	B
Study	Gascon 1989 [65]
Methods	Randomised, double-blind crossover study. Methods of randomisation not stated. Treatment periods of 6 weeks, total duration of 12 weeks. Results presented as combined data from both active treatment arms and both placebo arms. No washout period. Results first arms not stated.
Participants	12 patients out of a group of 23 patients with myotonic dystrophy (confirmed by well-established criteria). 1 drop-out because of normal relaxation time 6 patients were male, 6 female. Mean age not stated. Range from 18-55 years. Inclusion criteria: None stated. Exclusion criteria: None stated.
Interventions	Imipramine from 50-375 mg/day on the basis of plasma concentrations. Comparison with placebo.
Outcomes	Grip myotonia by measuring relaxation time after squeezing the examiner's two fingers for 2-3 seconds. Three successive timings of grip and percussion myotonia were taken, and the mean of these three was used as the patient's 'score'.
Allocation concealment	B

Characteristics of included studies (Continued)	
Study	Grant 1987 [66]
Methods	Randomised, single-blind crossover study. Method of randomisation not stated. Single centre Glasgow, Scotland. Treatment periods of 2 weeks. Total duration unclear. Results presented as combined data from both active treatment arms and both placebo arms. No washout period. Results first arms not stated.
Participants	10 patients without withdrawals. 10 patients with myotonic dystrophy. 6 patients were male, 4 female. Mean age 40.4 (SD not stated). Inclusion criteria: accepted clinical criteria and electromyographic criteria. Exclusion criteria: None stated.
Interventions	Nifedipine 10 mg 3x/day and nifedipine 20 mg 3x/day. Comparison treatment placebo.
Outcomes	Finger extension time of both hands measured as relaxation time after maximal voluntary contraction. The mean value of the first five extension times was measured.
Allocation concealment	B
Study	Kratz 1986 [68]
Methods	Randomised, double-blind crossover study. Methods of randomisation not stated. Single centre, Washington, D.C., USA. Treatment period not stated. Total duration not clear. Results presented as number of patients that improved. No insights in data. No washout period.
Participants	6 patients without withdrawals. 4 patients with myotonic dystrophy and 2 with myotonia congenita. Number of males, females, mean age and inclusion/exclusion criteria not stated.
Interventions	Mexiletine in doses up to 600 mg/day.
Outcomes	Grip strength. Relaxation time after making a fist, at room temperature and after the hand in icewater for 1 minute. Length of myotonic discharges.
Allocation concealment	B
Study	Kwiecinski 1992 [45]
Methods	Randomised, single-blind study. At beginning a crossover trial of phenytoin and placebo. Afterwards randomisation for disopyramide, tocainide or mexiletine. Methods of randomisation not stated. Single centre Poland. Treatment period of 4 weeks. Total duration unclear. Results for the crossover part of the study presented as combined data from both active treatment arms and both placebo arms. No washout period. Results first arms not stated. Overall results presented as outcome measures after 4 weeks of treatment.
Participants	30 patients with 2 withdrawals. 9 patients with myotonic dystrophy, 9 with dominant myotonia congenita and 12 with recessive myotonia congenita. 22 patients were male, 8 female. Mean age 31.8 years old (SD not stated). Inclusion criteria: accepted clinical criteria and electromyographic characteristics for different diseases. Exclusion criteria: None stated.
Interventions	Phenytoin 400 mg/day for 2 weeks and 600 mg/day last 2 weeks. Disopyramide 300 mg/day for 2 weeks and 600 mg/day last 2 weeks. Mexiletine 400 mg/day for 2 weeks and 600 mg/day last 2 weeks. Tocainide 800 mg/day for 2 weeks and 1200 mg/day last 2 weeks. Comparison treatment placebo.

Characteristics of included studies (Continued)	
Outcomes	Time needed to open eyes maximally after closure (Lid myotonia). Time needed to open hand after firm closure (Hand opening). Time needed to climb ten stairs (Stair-test). EMG relaxation time (Afterdischarge). Subjective responses. Each test was repeated three times at intervals of at least ten minutes. The mean value from three such measurements was taken as the time value for each test.
Notes	It is conspicuous that the sum of the number of patients in the different treatment groups of the randomisation part of the study exceeds the total number of included patients. Outcome measures were not measured in all patients (No reasons given).
Allocation concealment	B
Study	Lewis 1966 [67]
Methods	Randomised, double-blind crossover study. Randomisation arbitrarily by secretary. Single centre United Kingdom. Treatment periods of 3 weeks, total duration of 6 weeks. Results presented as combined data from both active treatment arms and both placebo arms. No washout period. Results first arm stated.
Participants	20 patients and 13 controls. 19 patients with myotonic dystrophy and 1 with myotonia congenita. Number of males and females not stated. Mean age not stated. Inclusion criteria: None stated. Exclusion criteria: None stated.
Interventions	Diazepam 5 mg 2x/day – 4x/day. Comparison treatment placebo.
Outcomes	Relaxation time with EEG surface electrodes on right forearm after 5 seconds of maximum voluntary contraction. Value was the mean of three measurements. Accurate progress notes with specific on grasp myotonia, percussion myotonia and toxic effects medication.
Notes	Great placebo effect; research into placebo pointed out that they contain 0.5 mg quinine sulphate per tablet.
Allocation concealment	B
Study	Leyburn 1960 [46]
Methods	Randomised, double-blind crossover study. Randomisation by statistician. Single centre United Kingdom. Treatment periods of three weeks, total duration twelve weeks. Results presented as individual data for different interventions and as combined data for treatment arms and placebo arms. No washout period. Results first arm not stated.
Participants	23 patients with 4 withdrawals. 16 patients with myotonic dystrophy and 4 with myotonia congenita. 9 patients were male, 11 female. Mean age not stated. Inclusion criteria: None stated. Exclusion criteria: None stated.
Interventions	Quinine (5 grain sugar coated tablets): 5 grains 2x/day first week and 5 grains 3x/day second and third week. Procainamide (0.25 g tablets): 0.5 g q.i.d. first week, 0.75 g q.i.d. second week and 1.0 g q.i.d. third week. Prednisone (5 mg tablets): 10 mg b.i.d. first throughout the three week period. Comparison treatment placebo.
Outcomes	Objective myotonia by measuring 3 times the after discharge with EMG and by measuring 3 times clinical relaxation time. The result is the average of all six measurements. Subjective opinion.
Allocation concealment	A

Characteristics of included studies (Continued)	
Study	Munsat 1967 [47]
Methods	Randomised, double-blind crossover study. Method of randomisation not stated. Single centre Los Angeles, USA. Treatment periods of three weeks, total duration 9 weeks. Results presented as combined data from four active treatment arms and both placebo arms. No washout period. Results first arm not stated.
Participants	9 patients without withdrawals. 7 patients with myotonic dystrophy and 2 with myotonia congenita. Number of males and females not stated. Mean age not stated. Inclusion criteria: Accepted clinical criteria, electromyography and muscle biopsy. Selected on the basis of intelligence and capability of being examined weekly and presented a spectrum of clinical involvement. Exclusion criteria: None stated.
Interventions	Diphenylhydantoin 100 mg 2x/day first week, 3x/day second week and q.i.d. third week. Procainamide 1 g 2x/day first week, 3x/day second week and 4x/day third week. Comparison treatment placebo.
Outcomes	Ergographic evaluation of hand grasp after five seconds of maximum voluntary contraction. Subjective report regarding efficacy or toxicity or both. Repeated ECG utilising standard leads.
Notes	Researcher could recognise medicine of patients by kind of adverse events.
Allocation concealment	B

Nine trials were found that compared active drug treatment with placebo for the treatment of myotonia, in a total of 103 patients with myotonic dystrophy type 1 and 30 patients with myotonia congenita.[45-47,63-68] One trial was found that compared two different drug treatments for the treatment of myotonia in 10 patients with myotonic dystrophy type 1.[69] On the basis of the title or the abstract a further 34 studies initially appeared to be eligible. However, by reading the full text of all potentially relevant studies 17 were non-randomised studies [48,54,70-83,101], ten were case studies [84-93] and a further seven did not have measures of myotonia as outcome measures (Table 2.2).[94-100]

Another study[102] is awaiting assessment because at the time of writing this review the trial results were not available in sufficient detail. We were informed about this study by contacting one of the disease experts in this field and read the abstract. This trial will be included in the next update of the review.

Trial design

Eight included trials were placebo-controlled, randomised, double-blind, crossover studies. The other two were placebo-controlled, randomised, single-blind, crossover studies.[45,66] All included trials were performed in a single centre and a total of 143 patients received treatment (active drug or placebo) over two weeks to six months. In one study the treatment period was separated by a 30-day period washout interval.[63] The other eight trials had no washout interval between the treatment periods.

The trial of Kwiecinski started as a crossover study.[45] Afterwards randomisation for three different study drugs took place. Remarkably the sum of the number of patients in the different treatment groups in the randomised part of the study exceeded the total number of included participants. An attempt to clarify this with the author was unsuccessful. We assume the second part of the study was not randomised until we receive evidence to the contrary.

Table 2.2 Characteristics of excluded studies

Study	Reason of exclusion
Alfonsi 2007 [84]	Case study
Backman 1990 [70]	Non-randomised study
Benstead 1987 [85]	Case study
Birnberger 1975 [71]	Non-randomised study
Brumback 1983 [72]	Non-randomised study
Cook 1984 [88]	Case study
Durelli 1982 [73]	Non-randomised study
Garai 1954 [89]	Case study
Geschwind 1955 [90]	Case study
Griggs 1977 [74]	Non-randomised study
Griggs 1978 [54]	Non-randomised study
Griggs 1989 [95]	No myotonia as outcome measure
Guilleminault 1978 [75]	Non-randomised study
Hughes 1991 [86]	Case studies
Jackson 1994 [87]	Case study
Karli 1974 [91]	Case study
Matsumura 2004 [101]	Non-randomised study
Mielke 1985 [76]	Non-randomised study
Milner-Brown 1990 [77]	Non-randomised study
Müller 1980 [78]	Non-randomised study
Orndahl 1986 [79]	Non-randomised study
Orndahl 1994 [96]	No myotonia as outcome measure
Pendefunda 1974 [92]	Case study
Pénisson-Besnier 2008 [97]	No myotonia as outcome measure
Ricker 1980 [80]	Non-randomised study
Rüdel 1980 [48]	Non-randomised study
Samaha 1964 [81]	Non-randomised study
Schneider 2003 [98]	No myotonia as outcome measure
Sechi 1983 [82]	Non-randomised study
Streib 1987 [93]	Case study
Sugino 1998 [83]	Non-randomised study
Tarnopolsky 2004 [94]	No myotonia as outcome measure
Vlachopapadopoulou 1995 [99]	No myotonia as outcome measure
Walter 2002 [100]	No myotonia as outcome measure

Participants

The trials did not provide baseline characteristics of the individual participants or of the two separated groups. Five trials did not give the baseline characteristics at all[46,47,64,67,68], the other trials gave the characteristics of the entire study population. Five trials included people with myotonic dystrophy only and five trials[45-47,67,68]

included participants with myotonic dystrophy as well as participants with non-dystrophic myotonic syndromes, in this case myotonia congenita. Five trials did not define explicit inclusion criteria.[46,65,67-69] Only Antonini et al. defined explicit exclusion criteria.[63] In this trial cardiac, ophthalmologic or urologic diseases were excluded. Since cardiac and ophthalmologic diseases are symptoms of myotonic dystrophy this trial probably included a selected group of patients.

Interventions

The regimens of treatment varied between studies (see characteristics of included studies). Most studies used drugs that block sodium channels (procainamide, disopyramide, phenytoin, quinine, tocainide and mexiletine) by which myotonia is diminished by reducing the level of depolarisation. Other drugs used were clomipramine, imipramine, taurine, nifedipine, diazepam and prednisone. It is hypothesised that the tricyclics (imipramine and clomipramine) act on the sympathetic nerve terminals to increase levels of norepinephrine, which exerts an inhibitory influence on skeletal muscle membranes by ß2-adrenoreceptor stimulation.[65,103] Taurine, an amino-acid, may affect cellular hyperexcitability by increasing membrane conductance of potassium and chloride.[64,73] All these types of drugs seem to act as membrane-stabilisers.

Outcome measures

The outcome measures used differed between trials. The most frequently used outcome measure was the clinical relaxation time in seconds. It was measured three seconds, two to three seconds, five seconds and three minutes after a maximum voluntary contraction (MVC).[63,65,67,69] Others did not specify the length of maximum voluntary contraction.[45,66,68]

The EMG relaxation time (after-discharge) in seconds after MVC was also used.[45,64,68] Additional ways of measuring relaxation time were used such as the use of EEG surface electrodes or an ergographic device.[47,67] Two trials used a mean score of three relaxation times and one used a mean score of five relaxation times after MVC.[65-67] Another trial used a mean score of six measurements consisting of three clinical relaxation times and three EMG relaxation times.[46]

Other outcome measurements were occurrence of percussion myotonia, percussion myotonia in seconds, eyelid myotonia in seconds after firm closure, occurrence of myotonic discharges induced by electrical stimulation of the median nerve, potassium chloride (KCl) loading test in mmol/litre for occurrence of myotonia, time to climb ten stairs (stair test) and subjective responses.[45,64,65,69]

Analysis

All trials were analysed on a per protocol basis (withdrawals were not included in the analysis) instead of an intention-to-treat basis.

Methodological quality of included studies

See Table 2.3.

Table 2.3 Methodological quality of included studies.

Study	Allocation concealment	Patient blinding	Observer blinding	Inclusion criteria	Exclusion criteria	Outcome measures
Antonini 1990 [63]	B	B	B	A	A	A
Durelli 1983 [64]	B	A	A	A	B	A
Finlay 1987 [69]	B	C	A	B	B	A
Gascon 1989 [65]	B	B	A	B	B	A
Grant 1987 [66]	B	B	B	A	B	A
Kratz 1986 [68]	B	B	B	B	B	A
Kwiecinski 1992 [45]	B	A	D	A	B	A
Lewis 1966 [67]	C	B	A	B	B	A
Leyburn 1960 [46]	A	B	B	B	B	C
Munsat 1967 [47]	B	A	C	A	B	A

A: adequate, B: unclear, C: inadequate, D: not done

The methodological quality assessment took into account allocation concealment, patient blinding, observer blinding, explicit inclusion and exclusion criteria and explicit outcome measures. We graded these items as: A: adequate, B: unclear, C: inadequate, D: not done. If the information was not available the item was graded as unclear. The scores of each trial are included in Table 2.3.

In all ten trials participants were randomised for crossover studies to either active treatment or placebo (or another active drug treatment). The allocation concealment was considered adequate in the study of Leyburn; a statistician randomised trial participants.[46] For the study of Lewis the allocation concealment was inadequate; the procedure was described as "arbitrary by secretary".[67] The other allocation concealments were unclear, because the method of randomisation was not explained.

Patient blinding was intended in at least nine trials. In only three trials the blinding was considered adequate.[45,47,64] In six trials the blinding was unclear because it was not described and in Finlay et al. the patient blinding was inadequate because participants could recognise the side effects of the medication, since these medication was previously used in a clinical setting.[46,63,65-69] Observer blinding was also intended in at least nine trials. Four trials were considered adequate for observer

blinding.[64,65,67,69] In one trial the observer could recognise the origin of the medication by the kind of adverse events.[47] Another single trial did not have observer blinding and the study of Grant et al. was designed as a randomised single-blind crossover study but it was unclear if the participants or the observers were blinded. [45,66] The other two studies were unclear. None of the trials recorded effectiveness of blinding.

We also graded the inclusion and exclusion criteria. This item is discussed under participants in the Description of studies section.
As expected there was no uniform outcome measurement. The explicit outcome measurements were considered adequate in eight trials. We considered the outcome measure of Leyburn et al. as inadequate, because it was the mean value of six measurements in which three were EMG relaxation times and three were clinical relaxation times.[46] It is difficult to give an explanation of the meaning of these values. Moreover, some studies took the mean of three to five relaxation times. It is likely that these times are shortened by the warm-up phenomenon.

Results

A total of ten single centre trials were included, in which 143 patients with myotonia were randomised in a single-blind or double-blind crossover study with a treatment period ranging from two weeks to six months. Twelve different drugs were used in those ten trials. Participants could be divided into 113 patients with myotonic dystrophy type 1 and 30 patients with myotonia congenita. Three studies were performed in the 1960s, six in the 1980s and one in the 1990s. In general the trials were small, with the participant numbers ranging from nine to thirty, and the methodological quality was poor. All ten included randomised crossover trials were based on a per protocol analysis which could result in an attrition bias. The data for an intention-to-treat analysis were not available.

The data analysis of Finlay et al. was inadequate.[69] The study only presented descriptive results. The individual continuous data were not stated and no statistical analysis was performed. The data of Kratz et al. were incomplete because we only have the information of the abstract (descriptive results).[68] Attempts to contact the author were unsuccessful. Lewis had a large placebo effect.[67] Research into the placebo tablets identified that they contained 0.5 mg quinine sulphate per tablet. This substance could be an effective treatment for myotonia, resulting in performance bias. For these reasons we were unable to use the data from these three trials.

Six studies were of crossover design without washout intervals.[45-47,64-66] Data were inappropriately presented in the form of combined results of both active treatment arms and both placebo arms. Since a washout interval was not incorporated there is a

strong possibility of a carry-over effect. Data from the first arms were not presented and four studies did not present data individually.[45-47,66] From these four studies three included both participants with myotonic dystrophy as well as myotonia congenita, without defining subgroups. For these reasons we were unable to use data from those four trials. We tried to contact the authors of the trials but have not yet been successful in obtaining the raw data. Two single studies gave data for some of our specified outcome measures and in spite of a carry over effect we will present these data.[64,65] For one study we can provide the results for the treatment of myotonia without any restrictions.[63] Because most trials included different diseases in the same trial without giving the individual data and used different drug treatments, meta-analysis was not possible.

Thus, it is only possible to present the data of three single studies for the treatment of myotonia in myotonic dystrophy.[63-65] We could not present potentially valuable data for the treatment of myotonia in non-dystrophic myotonic syndromes. For the study of Durelli et al. with a treatment period of six months it is only possible to present the data for our secondary outcome measure, the EMG relaxation time.[64] The EMG relaxation time after treatment with taurine was lower (average 0.58 seconds; SD 0.24) than both the baseline (average 1.33 seconds; SD 0.71) and after placebo (average 1.02 seconds; SD 0.36) (p-value<0.01; Student's t test). Taurine had no side effects.

Gascon et al. measured both left and right-hand relaxation times after imipramine and placebo.[65] Our primary outcome with the McNemar's test was significant for the right hand with an infinity odds ratio (95% CIs from binomial distribution 0.92 to infinity, p-value=0.025) and also significant for the left hand with an infinity odds ratio (95% CIs from binomial distribution 0.66 to infinity, p-value=0.046). The relaxation time was measured as a secondary outcome. Repeated measures of analysis of variance (ANOVAs) of these data revealed significant improvement of myotonia as measured by right grip ($F(2.20)=11.14$, p-value<0.001) and left grip ($F(2.20)=6.65$, p-value<0.01). The most important side effects of imipramine were dry mouth (8 out of 12 participants; 67%), dizziness (four out of 12; 33%), increased sweating (four out of 12; 33%), constipation (four out of 12; 33%), tremor (three out of 12; 25%), blurred vision (three out of 12, 23%) and diarrhoea (three out of 12, 23%).

The trial of Antonini et al. used clomipramine and had two washout intervals of thirty days, so the risk of carry-over effect was reduced.[63] They stated that there were no differences between patients receiving clomipramine in the first or second treatment period. The primary outcome measure of improvement of myotonia with the McNemar's test was not significant and showed an odds ratio of 3.00 (95% CIs 0.25 to 157.49) (p-value=0.32). The analysis of a secondary outcome measure with a paired t-test (crossover study) demonstrated that the mean relaxation time after

clomipramine (average 15.85 seconds, SD 9.44) was significantly shorter (p-value=0.02) than after placebo (average 22.54 seconds, SD 16.47). The study has no electromyographic relaxation time, stair test or presence of percussion myotonia as outcome measures. Minor side effects were drowsiness (six out of 15 participants; 40%), dry mouth (two out of 15; 13%), tiredness (two out of 15; 13%), hyperhydrosis (one of 15; 7%) and dizziness (one of 15; 7%).

In conclusion, it was only possible to calculate our primary outcome measure in this review for two studies.[63,65] This outcome was only significant for treatment with imipramine for myotonia in myotonic dystrophy.[65] Our secondary outcome measure of relaxation time could be calculated in the same two studies. Both imipramine and clomipramine showed a significant result in relieving myotonia in myotonic dystrophy. We could only provide data for the EMG relaxation time from the study with the treatment of taurine for myotonia in myotonic dystrophy.[64] This result was also significant. Meta-analysis was not possible.

The side effects of the other active drug treatments taken from the included trials were:
Mexiletine: 8% (2 of 24) epigastric distress prevented by taking the drug with food. Tocainide: 6% (1 of 18) lymphadenopathy and 11% (2 of 18) dizziness, anxiety and tremor. Diphantoin: 10% (3 of 30) skin rash, somnolence and mild ataxia. Disopyramide: 32% (7 of 22) dry mouth and blurred vision while taking high doses. Nifedipine: 20% (2 of 10) headache and lethargy while taking 3 doses of 20 mg and 10% (1 of 10) light T wave flattening or T wave inversion on the ECG. Procainamide: 39% (15 of 39) gastro-intestinal complaints. Quinine: 45% (9 of 20) mild and tolerable tinnitus, 30% (6 of 20) some degree of deafness and 5% (1 of 20) dull head without tinnitus. Prednisone: no side effects in three weeks. This is of course of little value in judging safety of steroid therapy as a long-term measure. Diazepam: 64% (7 of 11) sedation and 27% (3 of 11) of dizziness.

The tested drug treatments in this review varied in costs from EUR 2.29 per month (phenytoin) to EUR 23.67 per month (quinine).[104]

Discussion

Despite the fact that different drug treatments have been used to reduce symptoms of myotonia since 1936, very few good randomised crossover trials have been performed to study the effect of these treatments. Overall, the methodological quality of the studies considered was poor. Moreover, methods reported in original papers were not described in sufficient detail. Only one crossover trial had a washout interval and reported data from each treatment period. Clomipramine, studied in this

small trial, demonstrated a significant effect on the relaxation time in participants with myotonic dystrophy type 1. For more reliable results it is necessary to perform studies with a larger cohort. The other crossover trials did not have a washout interval and did not report data from each (or at least the first) treatment period separately. Four studies included participants with myotonic dystrophy type 1 as well as participants with myotonia congenita, without defining subgroups. For these reasons it was not possible to estimate the treatment effect of those four studies. Two other small studies indicated, despite a carry-over effect, a short-term effect of imipramine and a long-term effect of taurine on myotonia in myotonic dystrophy type 1.

In spite of the evidence (admittedly limited) for these three drugs mentioned above, these medications are probably not used very often in medical practice. Expert opinion still favours mexiletine. This is despite the lack of randomised controlled trials with mexiletine, although one is awaiting assessment.[102] In conclusion, better randomised crossover studies with a proper washout interval and clearly presented data from both arms and with clear separation of the different diseases associated with myotonia are necessary for further determination of an effective and safe treatment for myotonia.

The adverse events from randomised data are given in the results. Non-randomised data suggest serious side effects for tocainide and procainamide such as agranulocytosis and pancytopenia.[105-109] Other side effects of tocainide are diplopia, dizziness, nausea, tremor and anxiety.[48,76,80] For procainamide more than 50% of the patients had gastro-intestinal side effects and 33% complained of insomnia.[90] Three patients with myotonic dystrophy and treated with phenytoin or carbamazepine had cardiac side effects (ventricular tachycardia and atrioventricular block grade 1).[110] Reported side effects of acetazolamide were paraesthesias, anorexia, weight loss, renal failure, renal calculi, osteoporosis, and haematological and hepatic dysfunction.[54,74] In a non-randomised study with amitriptyline for myotonia six from the eight patients complained of a dry mouth and two had drowsiness. One participant had supraventricular tachycardia due to an adrenergic effect.[77] Verapamil for myotonia was tested in a non-randomised study in five people. One participant complained of dizziness with a first-degree heart block, another had transient nausea.[88]

The lack of appropriate trials and data is not the only difficulty in determining the treatment effect in myotonia. Difficulty also exists in the clinical assessment of myotonia. Although many outcome measures have been developed, until now no validated scale has been used with unanimous consent. Sansone et al. wrote an experimental protocol but also reported some unsolved problems.[56] Furthermore, myotonia can be dependent on temperature, physical effort, rest, food intake, pregnancy, and genotype. Therefore, it is difficult to standardise outcome measures

for myotonia. A technique to overcome some of the standardisation problems in measuring relaxation times, is the use of computerised protocols in which a computer program places cursors along the relaxation phase and calculates the relaxation times between these points.[1,57]

Another problem in determining the treatment effect of myotonia is the intriguing warm-up phenomenon (diminishing of myotonia after repetitive contractions). In ClCh this is probably the result of an improvement of both myotonia and transient paresis and in myotonic dystrophy and NaCh it is only the improvement of myotonia.[2] The exact pathophysiological mechanism of the warm-up phenomenon is unknown but the phenomenon could influence the degree of myotonia, especially when measuring relaxation times after repeated maximum voluntary contractions. The length and frequency of maximum voluntary contractions differed between studies which could influence the outcome measures. Furthermore, paramyotonia can occur, which is a worsening of myotonia after repetitive contractions (paradoxical myotonia). Myotonia is thus a symptom in different diseases. We excluded the diseases with no true myotonia (see background and type of participants) but when these are excluded there are still four groups left: (1) myotonic dystrophy type 1 (2) myotonic dystrophy type 2, (3) non-dystrophic ClCh, and (4) non-dystrophic NaCh. In general myotonia is a mild symptom in myotonic dystrophy and a much more serious symptom in non-dystrophic myotonic syndromes.

Paradoxically in our review only 30 patients with myotonia congenita were studied and the majority had myotonic dystrophy type 1, perhaps reflecting the higher prevalence of myotonic dystrophy. However, most patients with myotonic dystrophy do not seek treatment for their myotonia because it often is a relatively mild symptom compared to the other symptoms they suffer. They also may have an avoidant personality with 'avoidance' of medical treatment as part of their disease. Moreover, all studies including patients with a non-dystrophic myotonic syndrome, included patients with myotonic dystrophy as well. This causes a mixture of different diseases with different pathophysiologies, and the outcome measures were not analysed for the two disorders separately. For all the reasons mentioned above it would seem appropriate to perform different RCTs for the different kinds of myotonic syndromes. It is also unlikely that a single method of assessment is appropriate for each separate disease.

Finally, there is the lack of functional outcome measures. The most used functional outcome measure is the stair test (see last part of background), but only one study used this test. We recommend this test as a secondary outcome measure in future RCTs. Another possible functional test for future studies could be the chair test (time needed to stand up from a chair, walk around the chair and sit down again).

In conclusion, the best evidence for the treatment of myotonia in myotonic dystrophy type 1 is from single small studies with clomipramine, imipramine and taurine. Taurine did not have any side effects in nine people for six months. Clomipramine and imipramine have some side effects but seem to be safe treatments. We could not present valuable separate data for the treatment of myotonia in non-dystrophic myotonic syndromes. Based on the three single small randomised trials above in combination with clinical observations (subjective responses of the patients and expert opinion), some drugs have a potential effect in decreasing myotonia. To prove this hypothesis double-blind randomised controlled (multi-centre) trials have to be properly designed and performed for the different types of myotonic disorders. In the case of crossover trials a washout interval is recommended. Moreover, intention-to-treat analysis and appropriate analysis and presentation of the results are required.

Reviewers' conclusions

Implications for practice

The beneficial effect of active drug treatment for myotonia cannot be excluded and its use in certain patients with severe myotonia might be appropriate (for example in those in whom there is a clear impact on daily activities), however, there is a lack of randomised evidence to determine whether any drug treatment is safe and effective in the treatment of myotonia.

Implications for research

The clinical efficacy of drug treatment for myotonia has not yet been properly evaluated. Larger, well designed RCTs are needed to assess the efficacy and tolerability of drug treatment for myotonia.

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Chapter 3

Outline of this thesis

Outline of this thesis

The main objective of this thesis is to describe the phenotypic charecteristics of non-dystrophic myotonic syndromes (NDMs), including both chloride (ClCh) and sodium channelopathies (NaCh), on the basis of their genetics. Since the clinical features of NDMs were already described in the pre-genetic era, we will redefine these characteristics. The redefinition is evaluated in patients with a clinical, electrophysiological, and genetic diagnosis of NDM that were referred to our tertiary research centre during one full year.

Part I comprises the introduction to the research reported in this thesis. **Chapter 1** provides a general introduction of NDMs in which their history, pathophysiology, reclassification, ancillary investigations, differential diagnoses and treatment options are described. **Chapter 2** comprises a Cochrane review describing the results of the first systematic review of drug treatments for myotonia. **Chapter 3**, the current chapter, gives the outline of this thesis.

In **Part II, Chapter 4**, the genetic analyses of all the referred 54 NDM probands are reported. Furthermore, the value of in tandem analysis of *CLCN1* and *SCN4A* is evaluated. Based on earlier clinical criteria either *CLCN1* or *SCN4A* is sequenced. If no valuable mutations were detected in the first gene, the second gene was subsequently analysed. Besides the relevance of analysing *CLCN1* and *SCN4A* in tandem and mapping the responsible mutations in 54 probands, this study enabled us to compose genetically homogeneous groups of patients, thus facilitating closer scrutiny of the two NDM subtypes.

Part III of this thesis focuses on the redefinition of the clinical phenotypes of NDMs. **Chapter 5** describes three patients with a genotype-phenotype mismatch, underscoring the need for a redefinition of the clinical phenotypes of NDMs. Subsequently, in **Chapter 6**, the phenotypes of ClCh and NaCh are presented as based on classical standardised interviews and clinical bedside tests. The purpose of this study was to investigate the clinical pattern of myotonia in three different body regions (face, hands, legs) for the two channelopathies using widely practised neurological examination. The study also provides clinical rules of thumb for focussed genetic testing. In **Chapter 7** the health status of NDM patients is addressed, to our knowledge the first study to do so. It delineates the impact of this group of diseases on the patients' physical, psychological and social functioning. **Chapter 8** reports the results of muscle ultrasound measurements in combination with functional muscle parameters in genetically confirmed NDM patients in order to establish whether the various syndromes manifest structural muscle changes with functional consequences.

The final section, **Part IV, Chapter 9**, comprises the general discussion in which the findings as presented in this thesis are summarised and put into context. Moreover, remaining questions are addressed and future perspectives discussed.

Part II

Genetics

Chapter 4

In tandem analysis of *CLCN1* and *SCN4A* greatly enhances mutation detection in families with non-dystrophic myotonic syndromes

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Abstract

Objective
To optimise the genetic characterisation of NDMs in the Netherlands by analysing *CLCN1* and *SCN4A* in tandem.

Methods
All Dutch consultant neurologists and the Dutch Patient Association for Neuromuscular Diseases (Vereniging Spierziekten Nederland) were requested to refer patients with an initial diagnosis of a NDM for clinical assessment and subsequent genetic analysis over a full year. Based on clinical criteria, sequencing of either *CLCN1* or *SCN4A* was performed. When previously described mutations or novel mutations were identified in the first gene under study, the second gene was not sequenced. If no mutations were detected in the first gene, the second gene was subsequently also analysed.

Results
Underlying NDM mutations were explored in 54 families. Twenty percent (8/40) of our probands with suspected chloride channel myotonia showed no *CLCN1* mutations but subsequent *SCN4A* screening revealed mutations in all of them. All 14 probands in whom *SCN4A* was primarily sequenced showed a mutation. In total, *CLCN1* mutations were identified in 32 families (59%) and *SCN4A* in 22 (41%), resulting in a diagnostic yield of 100%. The yield of *mutation detection* was 93% with three recessive and three sporadic cases not yielding a second mutation. Among these mutations, 13 in *CLCN1* and three in *SCN4A* were novel.

Conclusion
The current results show that in tandem analysis of *CLCN1* and *SCN4A* affords a high-level of mutation ascertainment in families with a NDM.

Introduction

Non-dystrophic myotonic syndromes (NDMs) are a group of skeletal muscle disorders that have myotonia as their common feature, in reference to an abnormal muscle relaxation after voluntary or evoked muscle contraction. Myotonic discharges can be recorded by needle-electromyography (needle-EMG) from the skeletal muscles of these patients. Clinically, the NDMs are classified as dominant or recessive myotonia congenita (DMC; M. Thomsen [OMIM 160800] and RMC; M. Becker [OMIM 255700]), paramyotonia congenita (PC [OMIM 168300]) or potassium-aggravated myotonias (PAMs [OMIM 608390]). As suggested by Rüdel et al., PAM diagnosed without a potassium-loading test is referred to as a sodium-channel myotonia (SCM).[1]

In 1971 Bryant and Morales-Aguilera showed that the membrane resistance of myotonic goat muscle fibres was considerably elevated at rest, which was found to be due to a strongly diminished sarcolemmal chloride conductance.[2] The voltage-gated chloride channel concerned, was also shown to be involved in DMC as well as in RMC in humans.[3,4] Subsequent genetic studies demonstrated a linkage to the skeletal muscle chloride channel gene (*CLCN1* [OMIM 118425]), mapped to chromosome 7q35.[5,6] About 80 different *CLCN1* mutations have, so far, been associated with MC.[7] Meanwhile, in a second group of NDMs, impaired inactivation of voltage gated sodium channels was observed.[8] Various researchers later independently linked PC and PAM to the skeletal muscle sodium channel gene (*SCN4A* [OMIM 603967]), genetically mapped to chromosome 17q23-25.[9-11] To date, at least 30 different missense mutations have been identified in this gene.[12]

Although many mutations have been identified in *CLCN1*, 25 to 60% of the MC patients who were examined lacked any identifiable *CLCN1* mutation.[13-15] Comparable studies in patients with PC or PAM have so far not been performed. Although sequencing of the entire *CLCN1* increased the yield of putative myotonia-associated mutants, this was never able to account for all patients.[16] Limitations in mutation detection methods, genetic heterogeneity and additional modifying factors were proposed to explain the discrepancy.[13-16] Plassart-Schiess et al. postulated the incomplete dominance of some mutations with variable penetrance and expressivity as another compounding factor.[17] Additionally, patients with suspected DMC may show *SCN4A* mutations. The purpose of the present study was, therefore, to optimise the genetic characterisation of patients with NDMs in the Netherlands by in tandem analysis of *CLCN1* and *SCN4A*, as necessary.

Patients and methods

Proband selection

The current investigation comprised a cross-sectional, nationwide study. In March 2005, consultant neurologists across the Netherlands as well as the Dutch Patient Association for Neuromuscular Diseases (Vereniging Spierziekten Nederland), were requested to report patients with a clinical diagnosis of a NDM to our research group over a full year. All patients were subsequently contacted and those who responded positively were invited to the neurology outpatient clinic of the Radboud University Nijmegen Medical Centre for the proposed clinical assessment, needle-EMG and collection of blood samples for genetic analysis. Inclusion criteria were age over 18 years, a clinical diagnosis of a NDM according to established clinical criteria (Table 4.1), and myotonic discharges upon needle-EMG examination.[18]

Table 4.1 Clinical criteria for non-dystrophic myotonic syndromes.[18]

CHLORIDE CHANNELOPATHIES
Dominant myotonia congenita (DMC)
• Autosomal dominant inheritance • Age at onset from birth to early childhood • Myotonia, particularly after rest • Muscle function improves with continuing exercise (warm-up) • Myotonia fluctuates only slightly during lifetime, without progression
Recessive myotonia congenita (RMC)
• Autosomal recessive inheritance • Onset usually in the first decade of life • Myotonia, particularly after rest • Muscle function improves with continuing exercise (warm-up) • Often transient weakness after rest, improving with continuing exercise (warm-up) • Several years of progression, after which the condition stabilises
SODIUM CHANNELOPATHIES
Paramyotonia congenita (PC)
• Autosomal dominant inheritance • Onset from birth • Muscle function worsens with continuing exercise (paradoxical myotonia) • Paradoxical myotonia is especially common when muscles are exercised in low temperatures • Sometimes muscle weakness occurs when muscles are exercised in low temperatures
Potassium-aggravated myotonia (PAM)
• Autosomal dominant inheritance • Myotonia fluctuans: myotonia, which may fluctuate from day to day, is provoked by long periods of exercise (delayed-onset myotonia) • Myotonia permanens: persistent generalised myotonia, particularly in neck and shoulder muscles • Acetazolamide responsive myotonia congenita: myotonia fluctuates and, in addition, exercise induces muscle pain

Exclusion criteria included a clinical or genetic diagnosis of type 1 or type 2 myotonic dystrophy (dystrophic myotonic syndromes), a clinical or genetic diagnosis of primary periodic paralysis and unwillingness or inability to reduce or stop drug therapy for myotonia for the duration of the study. This latter criterion was added to optimise the clinical and electrophysiological evaluations of the myotonia. The study was approved by the Medical Ethics Committee of the Radboud University Nijmegen Medical Centre and all patients gave their written informed consent prior to their participation.

Preliminaries and procedure

CLCN1 and *SCN4A* were sequenced in tandem following our specially designed study strategy. The decision to sequence first *CLCN1* or *SCN4A* was based on established clinical criteria, which were independently verified for each patient by two authors (JT and GD). Disagreement was resolved by discussion. When previously described mutations were identified in the analysis of the first gene, we did not proceed with sequencing the second gene. In case of a suspected RMC in which we detected one mutation, we did not proceed with sequencing *SCN4A*. In case of novel mutations, their status and inheritance patterns were determined by clinical evaluations of first-degree relatives and by direct sequence analyses of their DNA. In addition, all novel missense mutations were screened in a control panel consisting of the DNA from 50 healthy Dutch individuals (100 chromosomes). In case no mutations were found in the first gene or novel mutations were not confirmed in first-degree relatives, we subsequently sequenced the second gene.

Mutation analysis

For each patient two 10-ml blood samples were collected in EDTA tubes. Genomic DNA was isolated from peripheral blood by the method of Miller et al. at the Leiden University Medical Centre and subsequently screened for mutations by direct sequence analysis of *CLCN1* and/or *SCN4A*. PCR analysis, purification of the PCR products (Millipore Multiscreen HTS PCR plates), sequencing (Big Dye Terminator Cycle Sequencer kit from Perkin-Elmer) and the final analysis (ABI3730) were performed as described previously with some minor modifications, indicated between brackets.[19,20] The primer sets used for amplification of *CLCN1* were made according to Lorenz et al. and primer sets designed for amplification of *SCN4A* can be found at http://www.lumc.nl/4080/DNA/SCN4A.html. [21]

Results

Study cohort

The recruitment procedure yielded a total of 113 probands, 23 of whom did not respond to initial contacts while 10 eventually refused participation without specifying their reasons. Nine were unable to participate due to transportation problems and three were unable to visit the hospital because of serious co-morbidity. Another 14 probands were excluded based on the following exclusion criteria: primary periodic paralysis (n=7), unwillingness/inability to reduce and stop drug therapy for myotonia (n=5), and no clinical diagnosis of a NDM with absence of myotonic discharges by needle-EMG (n=2). Accordingly, 54 probands with a clinically and electro-physiologically supported diagnosis of a NDM took part in this study. We, moreover, clinically and genetically examined 18 (20%) affected and 74 (80%) unaffected first-degree relatives of those probands for whom novel mutations or indistinct inheritance patterns were established. Table 4.2 shows the basic demographics for all 54 probands, and their 92 first-degree relatives.

Table 4.2 Basic characteristics of all eligible probands and their first-degree relatives (affected and unaffected) included in the *CLCN1* and *SCN4A* sequencing procedure.

	Probands (n=54)	**First degree affected family members (n=18)**	**First degree unaffected family members (n=74)**
Sex, n (%)			
Female	25 (46%)	7 (39%)	40 (54%)
Male	29 (54%)	11 (61%)	34 (46%)
Mean age yrs (SD)	43.1 (12.4)	49.9 (13.3)	54.8 (16.1)
Age yrs, range	19-71	26-76	18-87

Preliminaries and procedure data

For an overview of the results, see the flowchart depicted in Figure 4.1. In short, *CLCN1* was sequenced in 40 probands. For the remaining 14 probands, we first sequenced *SCN4A*. Mutations in *CLCN1* were identified in 32 probands. Subsequently, the remaining eight showed a mutation in *SCN4A*. For all 14 probands in whom *SCN4A* was the first to be sequenced, a *SCN4A* mutation was found. DNA analysis thus identified 32 (59%) probands with *CLCN1* mutations and 22 (41%) probands with *SCN4A* mutations, reflecting a 100% gene detection yield. However, the *mutation detection* yield, including homozygous recessives, was 93% (78 of 84) as in three recessive and three sporadic probands (worst case scenario) no second mutation was found.

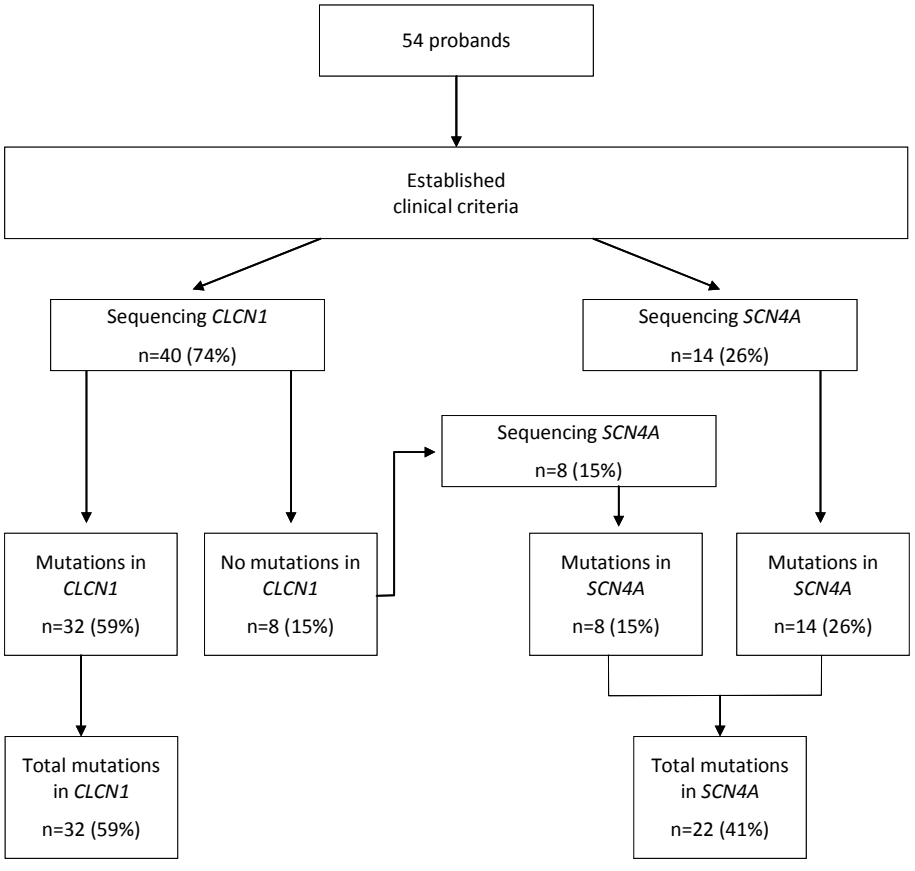

Figure 4.1 Flowchart presenting the numbers of probands (n, %) in whom the direct sequence analysis of *CLCN1* or *SCN4A* was performed. The flowchart also presents the results. Note that based on established clinical criteria in 40 probands *CLCN1* was sequenced first and that in 8 of these probands no mutation was identified. Also note that in 22 probands *SCN4A* was sequenced, 14 based on the mentioned clinical criteria and 8 based on the negative sequencing result of *CLCN1*. In total, *CLCN1* mutations were identified in 32 families and *SCN4A* in 22.

Mutation analysis data

CLCN1 mutations

In 32 probands, 21 different *CLCN1* mutations were identified, encompassing nine missense, five splice-site, four nonsense and three frameshift mutations (Table 4.3a). More than half (55%) of the mutations were detected in three exons (exon 3, 8 and 11). The remaining mutations were scattered across the entire chloride channel (Figure 4.2). Most of the 32 probands were compound heterozygote (n=17; Table

4.3b) and seven probands were homozygote, of whom four are known to result from consanguineous marriages. In eight probands only one mutation was detected of whom two showed an autosomal dominant inheritance (families 37 and 51; Table 4.3b), three were sporadic (families 10, 16 and 38; Table 4.3b) and for the other three, autosomal recessive inheritance seemed plausible (families 9, 27 and 41; Table 4.3b). Overall, the F413C missense mutation was the most frequently observed mutation (n=8; see Table 4.3a).

Table 4.3a Spectrum of *CLCN1* mutations identified in selected probands residing in the Netherlands.

Exon	Nucleotide change	Amino acid change	No of families with mutations	References
1	180+3A>T	splice-site donor	2	[34]
3	**302-1G>A**	**splice-site acceptor**	4	present study
3	**302-2A>C**	**splice-site acceptor**	2	present study
3	**385G>A**	**A129T**	2	present study
3	**411C>G**	**Y137X**	1	present study
4	501C>G	F167L	3	[13, 35]
5	**585_589del**	**K195fsX**	1	present study
7	**789delC**	**S264fsX**	4	present study
8	854G>A	G285E	5	[26]
8	**914G>A**	**G305E**	1	present study
10	**1065-2A>G**	**splice-site acceptor**	1	present study
11	**1167-10T>C**	**splice-site acceptor**	3	present study
11	1238T>G	F413C	8	[13, 35]
11	**1250A>G**	**E417G**	1	present study
13	1437_1450del	I479fsX	1	[13, 14, 34, 36]
13	1439C>T	P480L	1	[13, 37]
16	**1841A>T**	**K614M**	1	present study
17	**1938G>A**	**M646I**	3	present study
21	2419C>T	Q807X	1	[7, 22]
21	**2457C>A**	**C819X**	1	present study
23	2680C>T	R894X	3	[13, 14, 17, 32, 35]

No: number. Novel mutations are printed in bold.

In total, 13 of the 21 different mutations in *CLCN1* were newly identified mutations (62%) and comprised two nonsense, four splice-site, two frameshift and five missense mutations. The novel mutations 302-1G>A (n=4), S264fsX (n=4), M646I (n=3), 1167-10T>C (n=3), 3022A>C (n=2), and A129T (n=2) were detected more than once in our families and none of the novel missense mutations were detected in the 100 control chromosomes.

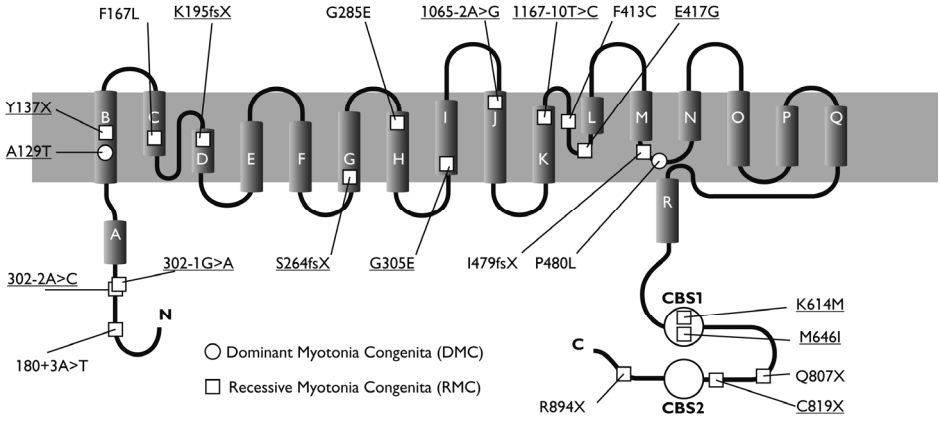

Figure 4.2 The sites of the mutations in the skeletal muscle chloride (ClC-1) channel as identified in the present study. Novel mutations are underlined, circles represent DMC (M. Thomsen) and squares RMC (M. Becker). The membrane topology of the ClC-1 channel is adapted from Dutzler et al.[45]

Novel *CLCN1* mutations

Y137X and C819X (recessive): Two novel nonsense mutations were identified. The Y137X mutation was detected in a recessive pedigree (family 26). The C819X was identified as the only mutation in two affected sibs of family 41. The healthy mother appeared to be the carrier of this mutation.

K195fsX (recessive): This novel frameshift mutation was identified in two patients of family 1 alongside the I479fsX mutation. A healthy sister, son and daughter appeared to be carriers of the K195fsX mutation.

S264fsX (recessive): This mutation was detected in two patients of family 6 alongside the novel missense mutation G305E. The father was carrier of the S264fsX mutation and the mother and an unaffected brother were heterozygous for the G305E mutation. Furthermore, the S264fsX mutation was the only recessive mutation in the sporadic probands of families 10 and 16. Two unaffected children and three unaffected siblings of family 10 and the mother of proband 16 were carriers of this mutation. Lastly, S264fsX was also identified in family 43 alongside the novel splice-site mutation 1065-2A>C.

Table 4.3b The mutational combinations in *CLCN1* as identified in the 32 probands with myotonia congenita.

Family nos	Consequences allele 1 / 2	Inheritance
01	**K195fsX** / I479fsX	recessive
02	**302-1G>A** / F413C	recessive
04 and 05	**302-1G>A** / **1167-10T>C**	recessive
06	**S264fsX** / **G305E**	recessive
07	**302-2A>C** / **302-2A>C**	recessive
08 and 22	F167L / F413C	recessive
09	F413C / -	probably recessive
10 and 16	**S264fsX** / -	sporadic
11	180+3A>T / **302-1G>A**	recessive
14	F413C / **1167-10T>C**	recessive
21	F413C / F413C	recessive
25 and 44	G285E / G285E	recessive
26	**Y137X** / F413C	recessive
27	F167L / -	probably recessive
29 and 32	R894X / R894X	recessive
37 and 38	**A129T** / -	dominant and sporadic
40	G285E / **E417G**	recessive
41	**C819X** / -	probably recessive
42	180+3A>T / **K614M**	recessive
43	**S264fsX** / **1065-2A>G**	recessive
46	G285E / **M646I**	recessive
47	E807X / E807X	recessive
48	**M646I** / R894X	probably recessive
51	P480L / -	dominant
54	**302-2A>C** / **M646I**	recessive
56	G285E / F413C	recessive

Nos: numbers. Novel mutations are printed in bold.

302-1G>A (recessive): This novel splice-site mutation was identified in a compound heterozygous state in all three affected sibs of families 2, 4 and 5 and in the recessive pedigree of family 11.

302-2A>C (recessive): This second novel splice-site mutation was observed in two families: homozygous in the proband of family 7, issue of a consanguineous marriage and compound heterozygous in a sporadic patient with a recessive mode of inheritance (family 54) alongside the newly identified missense mutation M646I.

1065-2A>C (recessive): The third novel splice-site mutation was identified in the proband of family 43 alongside the novel frameshift mutation S264fsX. The mother and an unaffected sister were carriers of the novel splice-site mutation and the father and two siblings were carriers of the novel frameshift mutation.

1167-10C>T (recessive): All affected sibs (n=5) of the recessive pedigrees 4 and 5 showed this novel splice-site mutation together with the novel 302-1G>A mutation.

Unaffected first-degree relatives in both families appeared to be carriers of one of the novel mutations. This mutation was also identified in a compound heterozygous state in a single patient of family 14. First-degree unaffected relatives carried one of the two mutations.

A129T (dominant): This missense mutation emerged in two families (37 and 38), i.e. one sporadic and one with a presumably dominant inheritance pattern. This could not be confirmed by DNA analysis because the affected father was deceased and the proband did not have children. *SCN4A* was also analysed retrospectively in both families and showed no mutations.

G305E (recessive): The second novel missense mutation was identified in family 6 and occurred in all affected family members alongside a novel recessive frame-shift mutation (S264fsX). The mutation was also identified in two unaffected siblings and the unaffected father.

E417G (recessive): This mutation was detected in the proband (E417G/G285E) of family 40. The unaffected mother was heterozygous for the E417G mutation; the father was deceased.

K614M (recessive): This fourth novel missense mutation was identified in family 42. The affected proband was compound heterozygous (K614M/180+3A>T). Both parents were deceased, while one brother was a carrier of the 180+3A>T and one sister was a carrier of the K614M mutation.

M646I (recessive): The M646I mutation was identified in three different families (46, 48 and 54). It was identified alongside the recessive missense mutation G285E, the nonsense mutation R894X and the novel splice-site mutation 302-2A>C, respectively. In family 48 the mutation was also detected in two affected sibs. In the recessive pedigree of family 54 the described mutations occurred in a single patient. In family 46 the father was a carrier of G285E, and the mother was deceased.

Clinical features of the probands with *CLCN1* mutations

All probands with *CLCN1* mutations showed obvious clinical signs of myotonia and 97% showed the warm-up phenomenon. About 15% of the probands showed transient paresis and muscle wasting, 48% only showed transient paresis and 37% showed neither transient muscle weakness nor muscle wasting. The probands with novel mutations did not show new clinical features compared with those having already known mutations.

SCN4A mutations

In 22 probands, 11 different missense mutations were identified (Table 4.4). Three mutations were located in domain I, one in domain II and six in the domains III and IV of the voltage-gated sodium channel, NaV1.4 (Figure 4.3). In 59% of the probands a mutation was identified in exon 22, of which the G1306V mutation was the most common (n=8). In total, three of the 11 different missense mutations were novel (27%), comprising two mutations in codon 250: L250V and L250P, and one in codon 689: L689F. Each of the three novel mutations was detected once and none of the novel missense mutations were detected in the 100 control chromosomes. All probands with novel mutations showed a phenotype mimicking Thomsen's disease. Therefore, DNA of these three probands was first sequenced for *CLCN1* mutations, but no mutations were identified.

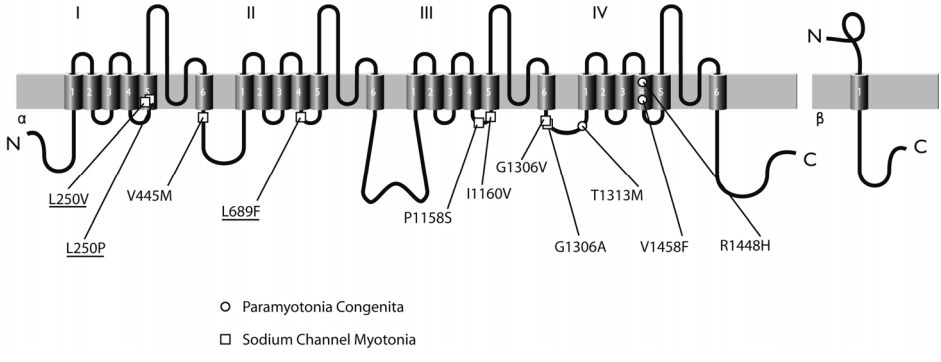

Figure 4.3 The sites of the mutations in the skeletal muscle sodium ($Na_V1.4$) channel as identified in the present study. Novel mutations are underlined, circles represent paramyotonia congenita (PC) and squares sodium-channel myotonias (SCM). The membrane topology of the $Na_V1.4$ channel is adapted from Jurkat-Rott et al.[33]

Table 4.4 Spectrum of *SCN4A* mutations identified in selected probands residing in the Netherlands.

Family nos	Exon	Nucleotide substitution	Amino acid substitution	No. of families with mutation	References
12	**6**	**748C>G**	**L250V**	1	present study
39	**6**	**749T>C**	**L250P**	1	present study
3, 18	9	1333G>A	V445M	2	[38]
36	**13**	**2065C>T**	**L689F**	1	present study
52	19	3472C>T	P1158S	1	[39]
49	19	3555A>G	I1160V	1	[40]
15, 24	22	3917G>C	G1306A	2	[10, 11, 34]
[19, 28, 30, 33, 45, 50, 53, 55]	22	3917G>T	G1306V	8	[10, 41]
13, 17, 23	22	3938C>T	T1313M	3	[41, 42]
35	24	4343G>A	R1448H	1	[43]
20	24	4372G>T	V1458F	1	[44]

Nos: numbers. Novel mutations are printed in bold

Novel *SCN4A* mutations

L250V (dominant): This missense mutation in the proband of family 12 is most probably a de novo mutation. Presumably, both his parents (deceased) were unaffected while his daughter and granddaughter were both affected and showed the same L250V mutation.

L250P (dominant): This mutation was identified in the proband of family 39. The affected father and brother in this family also showed this mutation. It was not detected in an unaffected brother and sister.

L689F (dominant): This mutation was detected as a de novo mutation in family 36. Both parents of the proband were unaffected and did not carry the mutation. The mutation was identified in both the proband and her affected daughter.

Clinical features of the probands with *SCN4A* mutations

All probands with *SCN4A* mutations showed obvious clinical signs of myotonia, especially in the eyelid muscles. Furthermore, probands with SCM, including the three probands with novel mutations, showed the warm-up phenomenon. The phenomenon was detected in the eyelid muscles (80%) as well as in the hand flexor muscles (80%). In contrast, probands with paramyotonia congenita showed paramyotonia in the eyelid muscles (40%) as well as in the hand-flexor muscles (75%). Finally, almost all probands with paramyotonia congenita showed an increase of paramyotonia or a flaccid paresis after cooling. Probands with SCM did not react to cooling.

Discussion

By sequencing *CLCN1* and *SCN4A* in tandem, we detected mutations in all our probands. The yield of *mutation detection* was 93%, with six cases (7%) not yielding a second mutation. This is a high percentage, especially compared to previous studies that identified *CLCN1* mutations in 40 to 75% of their MC patients, whereas analysis of *SCN4A* was not included.[13-15] Although the yield of our mutation detection was high, we failed to detect a second mutation in six probands. Possibly, deletions or other type of mutations deep in the intron or the promoter region of *CLCN1* may underlie the disease in these cases.[16]

Our strategy yielded 13 novel mutations in *CLCN1* and three in *SCN4A*. Although we did not perform *in vitro* studies, there are four lines of evidence affirming the suggestion that these mutations are pathogenic. First, eight of the 13 novel mutations in *CLCN1* were splice-site, frameshift or nonsense mutations, which are predicted to

eliminate channel function.[22] Second, none of the novel missense mutations occurred in the 100 control chromosomes. Thirdly, all original wild type amino acids at the sites of the missense mutations were well conserved across chloride or sodium channels of different species and/or among human chloride or sodium channels, and fourth, all but one (A129T) of the novel mutations were segregating with the disease.

Since the pathogenic status of missense mutations is less clear than the status of the other mutations, these mutations will individually be discussed by their location and conservation. First, the A129T mutation is located in transmembrane segment B of the human ClC-1 channel. A129 is a highly conserved amino acid across ClC channels of different species and is well conserved among the human plasma membrane ClC isoforms ClC-1, -2, -Ka and –Kb. Furthermore, A129T is in the vicinity of the already established M128V and S132C mutations. Both mutations segregated with the Thomsen phenotype.[23,24] M128V and S132C were both electrophysiologically characterised and showed a rightward shift in the current-voltage relationship, explaining their pathogenicity.[23-25] Second, the G305E mutation is located in transmembrane segment I of ClC-1. G305 is a highly conserved amino acid across ClC channels of different species and is well conserved among the human ClC isoforms ClC-1, ClC-2, ClC-Ka and ClC-Kb. G305E is in the vicinity of F307S, which was reported to drastically shift the voltage dependence of ClC-1 to positive potentials, preventing these channels from repolarising muscle action potentials efficiently.[26] Third, the E417G mutation is situated in the last codon of exon 11 and is therefore predicted to affect the splicing of this exon. However, further RNA studies are needed to explore this. Furthermore, E417, located in the linker between helix K and L of ClC-1, is highly conserved among ClC channels of different species. Fourth, the K614M mutation, conserved across ClC channels of different species but not among human ClC isoforms, and the L646I mutation, conserved across ClC channels of different species and among the human isoforms ClC-1 and ClC-2, are located in the β1 and β2-3 linker of the cystathionine β-synthase (CBS1) domain, respectively. Although the precise role of the CBS-domains is unknown, Estévez et al. suggested that mutations in this domain will influence the voltage-gated dependence of gating through the common gate.[27]

The L250P/V mutations in *SCN4A* are located in the membrane-spanning segment 5 of domain I of Nav1.4. Although in the vicinity of a benign polymorphism (S246L), L250 is highly conserved across $Na_V1.4$ channels of different species and among the α-subunits of human sodium channels $Na_V1.1$ to $Na_V1.8$.[28] Furthermore, the mutations were retrospectively absent in 200 control chromosomes. Finally, both probands with these mutations showed a definite phenotype of a NDM in the absence of other mutations in *CLCN1* or *SCN4A*, and both mutations were segregating with the disease.

The third novel *SCN4A* missense mutation (L689F), located in the linker between segment 4 and 5 of domain 2 in Nav1.4, is located at the same codon as the already established mutation L689I. This mutation was shown to cause in vitro effects of a hyperpolarising shift in the voltage dependence of activation causing hyperkalemic periodic paralysis.[29] The proband with the L689F mutation in our population showed a Thomsen-like phenotype without symptoms of weakness.

To exclude benign polymorphisms we tested 100 control chromosomes in accordance with the current best practice guidelines. Furthermore, the Leiden University Medical Centre analysed approximately 500 patients with suspected NDMs worldwide during the last five years and none of the detected variants were identified. Others mainly performed in *vitro studies* for the confirmation of novel mutations in one of these genes.[23,30,31] In the future such studies should also be done for the eight novel missense mutations we detected in our study.

In this study, we identified 21 different *CLCN1* and 11 different *SCN4A* mutations. Meyer-Kleine et al. also found a high number of different mutations in their German-based cohort.[13] In Scandinavian studies, three and eight different *CLCN1* mutations were detected, respectively.[16,32] In one of these studies the A513V, F413C and R894X mutations clearly predominated.[16] Thus, our study yielded a broad spectrum of mutations underlying NDMs in the Netherlands. We hypothesise that these findings may be attributable to the high population density in the Netherlands, especially when compared to the low population densities in Sweden, Norway and Finland.

Our analyses revealed DMC to be scarce in the Netherlands, which is in sharp contrast with the initial clinical diagnoses. In 20% of the patients, the initial referral diagnosis was DMC. However, only two of these patients were finally classified as DMC while the others proved to have SCM. All the probands with a SCM mutation showed a clinical picture of a generalised myotonia in combination with the warm-up phenomenon, mimicking Thomsen's disease.[33]

In conclusion, we have shown that in tandem analysis of *CLCN1* and *SCN4A* affords a high level of mutation ascertainment in families with a NDM. With this strategy we were able to identify 13 novel *CLCN1* and three novel *SCN4A* mutations. Moreover, it enabled us to confirm earlier suggestions that the prevalence of *SCN4A* mutations is higher than previously assumed.[18] Based on the results presented here, we feel safe in suggesting that our approach shows a great diagnostic potential and may offer optimal conditions for future genotype-phenotype studies.

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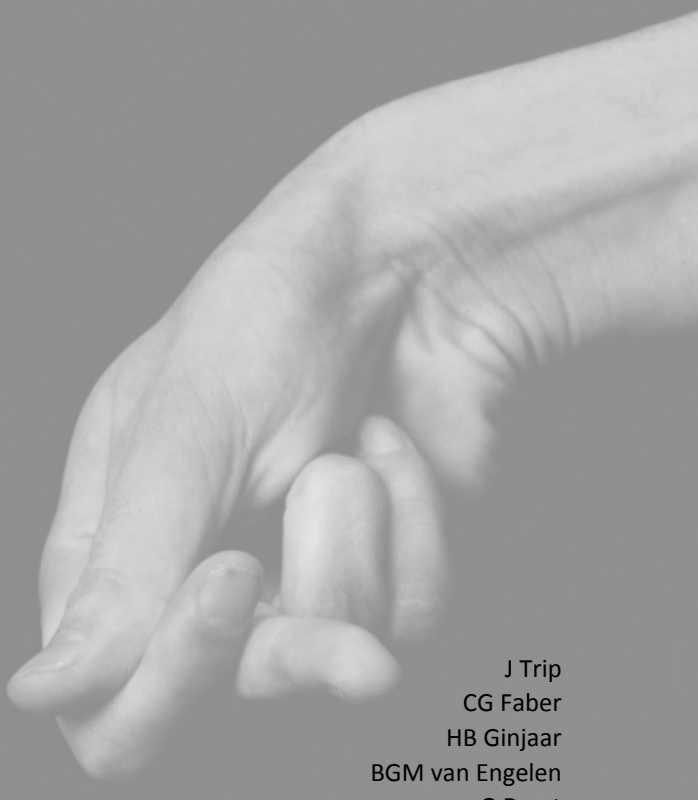
Part III

Phenotypical characteristics

Chapter 5

Warm-up phenomenon in myotonia associated with the V445M sodium-channel mutation



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Journal of Neurology 2007;254:257-258

Sirs: Myotonia is a clinical phenomenon consisting of uncontrolled temporary muscle stiffness after voluntary or evoked muscle contractions.[1] It is a cardinal symptom in non-dystrophic myotonic syndromes, including chloride (ClCh) and sodium channelopathies (NaCh). Myotonia typically occurs after a period of rest and decreases with continuing exercise, commonly referred to as the warm-up phenomenon. This is in contrast with what occurs in paradoxical myotonia, where muscle stiffness increases as a result of continuing exercise.

The warm-up phenomenon is an established clinical feature in ClCh, both in recessive myotonia congenita (RMC; M. Becker) as well as in dominant myotonia congenita (DMC; M. Thomsen). It has also been shown in limb muscles of patients with a NaCh.[1,2] By contrast, paradoxical myotonia has been established as a characteristic feature of NaCh.[3]

Thus, unlike the phenotypic homogeneity of ClCh, sodium-channel mutations are associated with a broad spectrum of clinical phenotypes.[4] Here we report three patients with a predominant and generalised warm-up phenomenon associated with the V445M missense mutation of the *SCN4A* gene encoding the alpha-subunit of the voltage gated sodium channel.

Three patients, from two families, with an autosomal dominant non-dystrophic myotonic syndrome and a predominant warm-up phenomenon were referred to our clinic. All patients had complaints of generalised muscle stiffness since birth and had earlier been diagnosed as Thomsen's disease, but recent chloride channel gene (*CLCN1*) mutation screening was negative. Clinically myotonia was generalised, severe and painful. All patients noticed aggravation of myotonia at cold temperatures and only one patient reported aggravation after eating potassium rich food. They all used sodium channel blockers (mexiletine 200 mg three times a day, procainamide 1000 mg three times a day and quinine 200 mg three times a day, respectively) with a good subjective effect. Needle EMG of all patients revealed myotonic discharges in all muscles investigated (left biceps muscle, right first interosseus muscle, right rectus femoris muscle, left tibialis anterior muscle and left orbicularis oculi muscle). We measured the warm-up phenomenon in three different muscle groups during a drug free period. The warm-up phenomenon of eyelid muscles and right hand flexor muscles was quantified by measuring the difference between two relaxation times - timed with a stopwatch -, i.e. after maximum voluntary contraction of three seconds following ten minutes of rest and after ten successive contractions. For leg muscles we compared the first and tenth trial on a 'chair test' in which the time required to rise from a standardised chair, to circumvent it and to sit down again was measured. DNA extracted from 20 ml of blood was screened for mutations in *SCN4A* by direct nucleotide sequence analysis.

All patients showed a marked generalised warm-up phenomenon (Table 5.1). Direct nucleotide sequence analysis of all three patients showed the same missense mutation (c.1333G>A; p.V445M) in *SCN4A*.

Table 5.1 Clinical relaxation times (in seconds) of three patients after the first (1^{st}) and tenth (10^{th}) maximum voluntary contraction of eyelid muscles (eyes), right hand flexor muscles (hand) and leg muscles (legs).

	Patient A	**Patient B**	**Patient C**
Age (years)/Sex	29/M	52/F	68/M
Eyes (1^{st})	3.77	25.00	17.46
Eyes (10^{th})	1.37	6.57	1.35
Hand (1^{st})	7.97	13.93	< 1.00 *
Hand (10^{th})	< 1.00 *	1.11	< 1.00 *
Legs (1^{st})	15.47	6.45	9.70
Legs (10^{th})	6.16	5.09	5.67

* no detection of clinical myotonia

The V445M sodium channel mutation was first reported by Rosenfeld et al. causing a painful myotonia congenita.[2] The warm-up phenomenon was mentioned in one patient. Here we report three patients with a predominant and generalised (eyelid muscles, right hand flexor muscles and leg muscles) warm-up phenomenon in association with the same V445M sodium-channel mutation. Although quantitative computer analysis for the warm-up phenomenon of a single muscle group has already been developed, we used clinical bedside tests in order to be able to measure this phenomenon in three various muscle groups.[5]

For a definite molecular diagnosis clinicians should be aware that a predominant and generalised warm-up phenomenon may be found in ClCh, but also in NaCh. A definite diagnosis is important for informing patients about their disease, orienting therapy and genetic counselling.[4] Thus, in cases with severe, painful myotonia in combination with a clear warm-up phenomenon, we recommend screening of *SCN4A* with particular attention for the V445M mutation.

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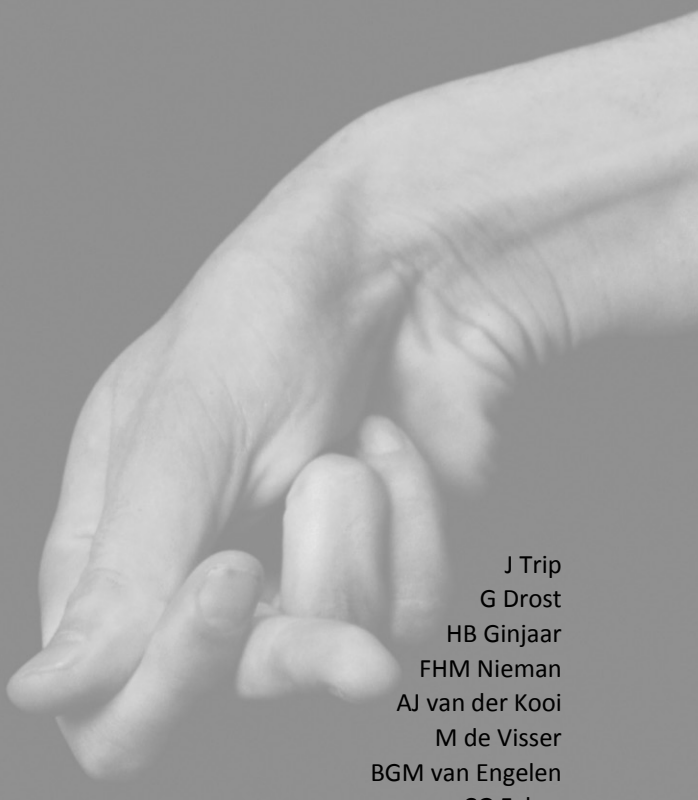
Chapter 6

Redefining the clinical phenotypes of non-dystrophic myotonic syndromes



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Abstract

Objective
To redefine phenotypical characteristics for both chloride (ClCh) and sodium channelopathies (NaCh) in non-dystrophic myotonic syndromes (NDMs).

Methods
In a cross-sectional, nationwide study, standardised interviews and clinical bedside tests were performed in 62 genetically confirmed NDM patients, 32 ClCh and 30 NaCh.

Results
Standardised interviews revealed that ClCh reported a higher frequency of muscle weakness (75 versus 36.7%; p<0.01), the warm-up phenomenon (100 versus 46.7%; p<0.001), and difficulties in standing up quickly (90.6 versus 50%; p<0.001), running (90.6% versus 66.7; p<0.05), and climbing stairs (90.6 versus 63.3%; p=0.01). Patients with NaCh reported an earlier onset (4.4 versus 9.6 years; p<0.001), and higher frequencies of paradoxical (50.0 versus 0%; p<0.001), and painful myotonia (56.7 versus 28.1%; p<0.05). Standardised clinical bedside tests showed a higher incidence and longer relaxation times of myotonia in the leg muscles for ClCh (100 vs. 60%; mean duration of chair tests 12.5 vs. 6.3 seconds; p<0.001), and in eyelid muscles for NaCh (96.7 vs. 46.9%; mean relaxation time of 19.2 vs. 4.3 seconds; p<0.001). Transient paresis was only observed in ClCh (62.5%) and paradoxical myotonia only in NaCh (30.0%). Multivariate logistic regression analyses allowed us to propose clinical guidelines for genetic testing.

Conclusion
This study redefined the phenotypical characteristics of NDMs in both ClCh and NaCh. The clinical guidelines that we proposed may help clinicians to perform focussed genetic analysis of either *CLCN1* or *SCN4A*.

Introduction

Non-dystrophic myotonic syndromes (NDMs) are a heterogeneous group of skeletal muscle disorders caused by mutations in genes encoding the skeletal muscle chloride (*CLCN1*) or sodium channel (*SCN4A*). Mutations in *CLCN1* are responsible for recessive and dominant myotonia congenita (RMC and DMC), and mutations in *SCN4A* for paramyotonia congenita (PC), potassium-aggravated myotonias (PAM), and hyperkalemic periodic paralyses with myotonia (HYPP).[1] As suggested by Rüdel et al., PAM diagnosed without a potassium-loading test is referred to as a sodium-channel myotonia (SCM).[2]

Diagnosis of the various types of NDMs was originally based on clinical characteristics only. Thomsen was the first to describe DMC, distinguishing the stiffness (myotonia), reduction of stiffness through repetitive muscle contractions (warm-up phenomenon), and the dominant inheritance of the disease.[3] In 1957 Becker described a recessive form with a more generalised myotonia in combination with transient paresis.[4] Both symptoms also improved with sustained exercise (warm-up phenomenon).[5] In contrast, myotonia in PC worsens with sustained exercise (paradoxical myotonia). Furthermore, in PC a flaccid paresis may be elicited by cold or long periods of exercise.[6] PAM is clinically characterised by potassium sensitivity of myotonia and by unusual features as fluctuations in myotonia (myotonia fluctuans), permanent myotonia (myotonia permanens) or acetazolamide-responsive MC.[7,8] HYPP shows attacks of generalised muscle weakness with myotonia.[9]

Since genetic testing became available, several authors have reported genotype-phenotype mismatches.[10-12] For example, the warm-up phenomenon was assumed to be a specific symptom for chloride channelopathies. However, instead of the expected chloride channel defect, a mutation in the skeletal muscle sodium channel was found in some families with the warm-up phenomenon.[10-12] The present study aims to redefine clinical phenotypes of NDMs segregated by chloride (ClCh) and sodium channelopathies (NaCh). To this end, we conducted standardised interviews and clinical bedside tests in a group of genetically confirmed patients with NDMs.

Patients and methods

Patients

All neurologists across the Netherlands as well as the Dutch Patient Association for Neuromuscular Diseases (Vereniging Spierziekten Nederland) were requested to report all patients with NDMs to our research group for a full year. All reported patients aged 18 years and over were invited to the neurology outpatient clinic of the

Radboud University Nijmegen Medical Centre and seen once for clinical assessment, needle-EMG, and collection of blood samples for genetic analysis. Inclusion criteria were a clinical diagnosis of a NDM according to established clinical criteria and myotonic discharges upon needle-EMG examination.[13] Exclusion criteria were a clinical or genetic diagnosis of myotonic dystrophy type 1 or type 2, a clinical or genetic diagnosis of primary periodic paralysis without clinical signs of myotonia, serious co-morbidity, absence of mutations by direct sequence analysis in *CLCN1* or *SCN4A*, and unwillingness or inability to temporarily stop drug therapy for myotonia. The study was approved by the medical ethics committee of the Radboud University Nijmegen Medical Centre, and all participating patients gave their written informed consent prior to the study.

Genetic analysis

Genomic DNA was isolated from peripheral blood at the Leiden University Medical Centre and subsequently screened for mutations by direct sequence analysis of *CLCN1* and *SCN4A*.[14] Primer sets designed for amplification of *CLCN1* or *SCN4A* can be found at http://www.lumc.nl/4080/DNA/CLCN1.html or SCN4A.html, respectively.

Phenotype characterisation

All clinical evaluations were conducted by the same examiner (JT) who had been amply trained in the standardised examination of patients with NDMs.

Standardised interview

A standardised interview was conducted to establish demographic and general clinical aspects (age, sex, age of onset, duration of symptoms, duration prior to diagnosis, use and duration of drug therapy and anesthesia-related adverse events), specific clinical features such as myotonic features (presence, provoking factors, pattern, frequency and severity (Numerical Rating Scale (NRS), range 1-10), presence of painful myotonia, severity of the pain (NRS, range 1-10), course of the disease, other neuromuscular features (presence of muscle weakness), disabilities (disability interfering with household and employment), and finally, the patient's ability to walk, climbing stairs, stand-up quickly, run, and play sports.

Standardised neuromuscular examination (clinical bedside tests)

A standardised neuromuscular examination was performed to determine the presence of myotonia in eyelid, hand flexor, and leg muscles. Furthermore, presence or absence of the warm-up phenomenon, paradoxical myotonia, and transient paresis was tested. All tests were performed in predefined, standardised positions and environments (20 °C) at the same time of the day. Before the study, a trained clinical

team pilot tested the draft standardised neuromuscular examination and also repeated this is in several patients.

Myotonia assessment

Clinical tests for myotonia were performed after ten minutes of rest. Action myotonia in eyelid muscles and right-hand flexor muscles was determined after a maximum voluntary contraction of three seconds, which was initiated and the maximum possible contraction maintained on instruction from the examiner, after which the patients were asked to open their eyes or fist as quickly as possible. Action myotonia in leg muscles was evaluated during a modified 'chair-test', in which the time required to rise from a standardised chair, to walk around it and to sit down again was measured.[15] Percussion myotonia in the hands and legs was determined with a blow of the percussion hammer on the right thenar and right quadriceps muscle, respectively. Finally, myotonia of each muscle group was scored as positive if either action myotonia or percussion myotonia or both were present.

Assessment of the warm-up phenomenon and paradoxical myotonia

Relaxation times of the eyelid muscles and right-hand flexor muscles as well as performance of the chair tests were timed by stopwatch. The tests were conducted after ten minutes of rest and after ten successive contractions or, for the chair test, ten consecutive cycles. The warm-up phenomenon was defined as a reduction of the relaxation interval following the contractions or chair-test cycles with at least one second. Conversely, paradoxical myotonia was defined as an increase of the relaxation interval with at least one second.

Assessment of transient paresis

Presence of transient paresis was evaluated by manually testing the left biceps during isometric muscle force maintained during five seconds. If during this 5-sec interval the Medical Research Council (MRC) score fell below 5, the patient was encouraged to do warm-up exercises (i.e. 10 strong, successive 10 second contractions with the examiner exerting counterforce).[16] If following the warm-up exercises the MRC-score had increased by minimally one point, transient paresis was recorded as positive and, if it had not, as negative.

Statistical analysis

The clinical and genetic data were recorded as independent variables in an SPSS database. All data analyses were performed using SPSS version 15.0 for windows (SPSS Inc, Chicago, IL, USA). To compare ClCh with NaCh, DMC with RMC, and PC/HYPP with SCM, we applied the Mann-Witney-U test for independent groups for

continuous variables and the chi-squares test for categorical variables. For the distinction between ClCh and NaCh we used multivariate logistic regression analysis. We regarded age, gender, and all variables obtained by the standardised neuromuscular examination as possible predictors. To find the best-fitting predictive model we used backward elimination and log-likelihood chi-squares. Subsequently, we searched for a reduced regression model including only predictors with a log-likelihood chi-squares p-value lower than 0.10. Next, first-order interactions between all pairs of predictors remaining within the reduced model were tested by forward selection with log-likelihood chi-squares. Finally, we tried to construct a practical instrument for the distinction between ClCh and NaCh in daily clinical practice. A p-value of less than 0.05 was considered to be statistically significant.

Results

Patient demographics and genetic data

The 1-year recruitment period yielded a total of 158 patients of whom 110 had been reported by neurologists and 48 by the Dutch Patient Association for Neuromuscular Diseases. Of the former contingent, 38 ultimately refused participation without specifying their reasons, and 23 patients of the latter group were non-responders. Nine patients were unable to participate due to transportation problems and 26 patients were excluded because of periodic paralysis without clinical myotonia (n=7), unwillingness or inability to temporarily stop drug therapy for myotonia (n=5), misdiagnosis (n=2), or serious co-morbidity (n=12). Finally, we included 62 patients with a clinical, electromyographical and genetically confirmed diagnosis of a NDM, originating from 48 unrelated families. Thirty-three were men (53.2%) and 29 women (46.8%) and their mean age was 42.3 years (± 11.9 years, range 19-68). Direct sequence analysis showed *CLCN1* mutations in 32 and *SCN4A* mutations in 30 patients.[14]

Phenotype characterisation

Standardised Interview

Differences between ClCh and NaCh

Table 6.1 shows the prevalence of clinical aspects that were statistically significantly different between ClCh and NaCh.

Table 6.1 Prevalence of clinical aspects derived from the standardised interview that were statistically significantly different between chloride (ClCh) and those with sodium channelopathies (NaCh).

	ClCh	NaCh	p-value
Mean age in yrs (SD), range	45.7 (10.6), 23-60	38.7 (12.3), 19-68	0.02
Mean age of onset in yrs (SD), range	9.6 (7.3), 0-31	4.4 (7.0), 0-36	<0.001
Mean duration to diagnosis in yrs (SD), range	12.0 (10.4), 0-33	7.7 (11.9), 0-51	0.04
Sleep deprivation, provoking factor of myotonia, n (%)	11 (34.4)	19 (63.3)	0.02
Decrease myotonia after repetitive contractions, n (%)	32 (100)	15 (50.0)	<0.001
Increase myotonia after repetitive contractions, n (%)	0 (0)	15 (50.0)	<0.001
Presence of muscle weakness, n (%)	24 (75)	11 (36.7)	0.002
Presence of painful myotonia, n (%)	9 (28.1)	17 (56.7)	0.02
Difficulty in climbing stairs, n (%)	29 (90.6)	19 (63.3)	0.01
Difficulty in standing-up quickly, n (%)	29 (90.6)	15 (50.0)	<0.001
Difficulty in running, n (%)	29 (90.6)	20 (66.7)	0.02

Differences within ClCh and NaCh

Within the ClCh there were no statistically significant differences between RMC and DMC. Within the NaCh group only muscle weakness showed a significant difference between PC and SCM (PC 85.7% versus SCM 21.7%; p=0.002).

Similarities between ClCh and NaCh

In addition to the discriminating variables, most patients mentioned daily complaints (ClCh: 96.9% and NaCh 83.3%) and myotonia was reported as severe (ClCh: 71.9% ≥5 versus NaCh: 67.7% ≥5) with symptoms having increased during their lives. Furthermore, patients with painful myotonia rated their pain as severe (ClCh: 77.8% ≥5 versus NaCh: 76.5% ≥ 5). Muscle contractions (95.2%), low temperatures (88.7%), and stress and emotions (61.3%) were the main provoking factors for myotonia. In the female patients both menstruation and pregnancy tended to aggravate the symptoms (menstruation: ClCh: 25% and NaCh: 41%; pregnancy: ClCh: 40% and NaCh: 80%). About 30% of the patients (ClCh: 31.3% and NaCh: 26.7%) had been taking drugs to combat myotonia for a prolonged time (mean duration of drug therapy 10.1 vs. 15.3 years). Fifty percent used mexiletine, the other half carbamazepine, fenytoine, tocainide, procainamide, or flecainide. In each group about one third of the patients was fully or partially disabled (ClCh: 34.4 and NaCh: 33.3%). Finally, complications during anesthesia were reported in 21.7% in ClCh and in 23.3% in NaCh: two patients with a NaCh showed myotonia in tongue and throat muscles, which hampered their intubation. One patient with a NaCh showed abundant myotonia in his abdominal muscles, which made it necessary to cancel surgery. Two patients with a ClCh were excessively fatigued for a prolonged time, and eight patients, four ClCh and four NaCh, experienced an excessive increase of their myotonia after surgery.

Standardised neuromuscular examination

Differences between ClCh and NaCh

Table 6.2 shows the results of the standardised neuromuscular examinations. Eleven of the 16 variables differed statistically significantly between the two NDM groups. Most noticeable were the higher occurrence and longer relaxation times of myotonia in the leg muscles for ClCh and in the eyelid muscles for NaCh. Furthermore, paradoxical myotonia was exclusively present in the NaCh and transient paresis in ClCh.

Table 6.2 Clinical features obtained from the standardised neuromuscular examinations in patients with chloride (ClCh; n=32) and sodium channelopathies (NaCh; n=30).

	ClCh	**NaCh**	**p-value**
Action myotonia right-hand flexor muscles, n (%)	29 (90.6)	27 (90.0)	0.93
Action myotonia leg muscles, n (%)	29 (90.6)	7 (23.3)	<0.001 *
Percussion myotonia right abd. poll. brevis, n (%)	28 (87.5)	26 (86.7)	0.92
Percussion myotonia right quadriceps, n (%)	27 (84.4)	17 (56.7)	0.02 *
Myotonia in eyelid muscles, n (%)	15 (46.9)	29 (96.7)	<0.001 *
Myotonia in right-hand flexor muscles, n (%)	29 (90.6)	29 (96.7)	0.32
Myotonia in leg muscles, n (%)	32 (100)	18 (60.0)	<0.001*
Mean relaxation time of patients with myotonia in eyelid muscles after 1 contraction in sec (SD), range	4.3 (3.7), 0.7-13.1	19.2 (14.1), 1.0-54.5	<0.001 *
Mean relaxation time of patients with myotonia in eyelid muscles after ten contractions in sec (SD), range	0.7 (0.8), 0-2.9	14.1 (18.0), 0-79.9	<0.001 *
Mean relaxation time of patients with myotonia in hand muscles after 1 contraction in sec (SD), range	4.7 (3.9), 1.1-17.5	5.6 (6.5), 0.7-30.7	0.90
Mean relaxation time of patients with myotonia in hand muscles after 10 contractions in sec (SD), range	1.1 (1.2), 0-5.9	7.9 (19.3), 0-99.0	0.01 *
Mean duration of the chair test after rest in sec (SD), range	12.5 (5.7), 5.2-29.9	6.3 (2.2), 4.0-15.5	<0.001 *
Mean duration of the chair test after 10 cycli in sec (SD), range	7.0 (2.1), 4.3-15.3	5.7 (1.0), 4.0-8.9	<0.01 *
Warm-up phenomenon, n (%)	30 (93.8)	23 (76.7)	0.05
Paradoxical myotonia, n (%)	0 (0)	9 (30.0)	<0.001 *
Transient paresis, n (%)	20 (62.5)	0 (0)	<0.001 *

* p<0.05

Differences within ClCh and NaCh

In ClCh transient paresis showed no differentiation between RMC (66.7%) and DMC (0%) (p= 0.13). Furthermore, there were no other differentiating variables between the two MC types. In NaCh the presence of myotonia in the leg muscles (p=0.05), action myotonia (p=0.10), and percussion myotonia of the right quadriceps muscle (p=0.09) showed a trend for differentiation between SCM and PC (Table 6.3). However, only the warm-up phenomenon contributed statistically significantly to the differentiation between SCM and PC (Table 6.3).

Table 6.3 Clinical features as obtained from the standardised neuromuscular examinations in the patients with paramyotonia congentia (PC; n=7) and sodium-channel myotonias (SCM; n=23).

	PC	SCM	p-value
Myotonia in leg muscles, n (%)	2 (28.6)	16 (69.6)	0.05
Action myotonia leg muscles, n (%)	0 (0%)	7 (30.4)	0.10
Percussion myotonia right quadriceps, n (%)	2 (28.6)	15 (65.2)	0.09
Warm-up phenomenon, n (%)	2 (28.6)	21 (91.3)	0.001 *

* p<0.05

Similarities between ClCh and NaCh

Both groups exhibited a high incidence and similar severity of myotonia in the hand-flexor muscles, and both displayed the warm-up phenomenon.

Practical instrument of parameters for the distinction between ClCh and NaCh

Tables 6.4a and 6.4b show the results of the final logistic regression model.

Table 6.4a Results of the final logistic regression model with type of channelopathy as the dependent outcome measure (n=62).

	B	S.E.	Wald	df	Sig.	Oddsratio	95% C.I. for OR Lower - Upper
Predictors							
Myotonia in eyelid muscles	4.47	1.22	13.49	1	<0.001	87.00	8.02 -943.35
Transient paresis	-22.69	7812.13	0.00	1	0.998	0.00	0.00 -
Constant	-2.20	1.05	4.35	1	0.037	0.01	

Table 6.4b Test results of myotonia in eyelid muscles and transient paresis in the final model.

	Model Log Likelihood	Change in -2Log Likelihood	df	Sig. of Change
Predictor				
Myotonia in eyelid muscles	-25.13	23.84	1	<0.001
Transient paresis	-32.09	37.77	1	<0.001

The resultant model only comprises myotonia in eyelid muscles and transient paresis. The log-likelihood chi-squares of this model are 59.47 by 2 df (p<0.001), and the ROC area-under-the-curve parameter is 0.947 (asymptotic 95% CI: 0.884-1.000). Based on these results quite simple rules for an effective and practical instrument to distinguish ClCh from NaCh can be described: if both eyelid myotonia and transient paresis are absent OR if transient paresis is present (regardless of the absence or presence of eyelid myotonia) a ClCh is most likely (See Table 6.5). Conversely, if eyelid myotonia is present and transient paresis is absent a NaCh is the most likely conclusion (Table 6.5).

Table 6.5 Cross table showing the relationship between the clinical bedside tests (transient paresis and myotonia in eyelid muscles) and type of channelopathy (chloride channelopathies (ClCh) and sodium channelpathies (NaCh); n=62).

	ClCh	NaCh
TESTS		
Myotonia in eyelid muscles absent AND Transient paresis absent OR Transient paresis present	29	1
Myotonia in eyelid muscles present AND Transient paresis absent	3	29

Sensitivity and specificity of this test for ClCh are 90.6% and 96.7%, respectively. Thus, the combination of the individual results and their multivariate regression analyses showed that the presence or absence of paramyotonia, transient paresis, and eyelid myotonia discriminate between ClCh and NaCh (Figure 6.1).

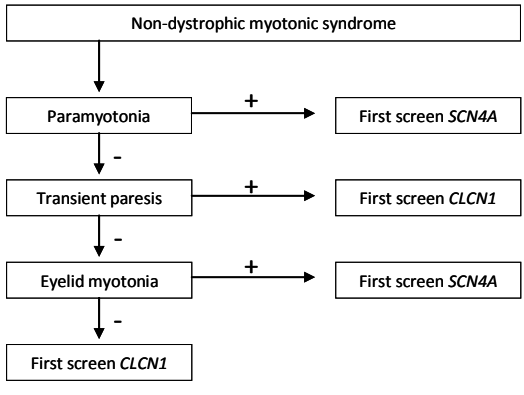

Figure 6.1 Clinical guideline for genetic testing based on the standardised clinical bedside tests.

Discussion

In this systematic cross-sectional study with a thorough standardised clinical protocol and a robust methodology we were able to redefine the clinical phenotypes of NDMs. Using one of the largest genetically confirmed NDM cohorts of both ClCh and NaCh, we showed novel genotype-phenotype correlations and confirmed earlier ones. Before, Becker and colleagues had thoroughly studied the clinical picture of NDMs in the pre-genetic era.[17,18] Fialho et al. recently reported a large cohort of ClCh, while

Matthews et al. described a large cohort of NaCh.[19,20] This study included both ClCh and NaCh, allowing a direct comparison of the two phenotypes.

The main result of our study is the high frequency and severity of myotonia in the eyelid muscles in NaCh and in the leg muscles in ClCh. Furthermore, we confirmed that transient paresis is a unique clinical feature for ClCh and paradoxical myotonia for NaCh. Interestingly, the warm-up phenomenon was demonstrated in both NDM groups. Another remarkable finding is that 28.1% of the ClCh patients and 56.7% of those with NaCh experienced painful myotonia, whereas myotonia is usually considered painless.[1]

The distribution pattern of myotonia in our study differs between ClCh and NaCh. Original reports described the distribution patterns of MC as generalised with leg muscles often severely affected.[17] Eulenburg, Streib and Rüdel et al. reported a predilection of myotonia in PC for facial, tongue, throat, and hand muscles.[2,6,21] In Matthews' genetically confirmed PC population 26 of the 32 patients reported myotonia in the face and the upper limbs.[20] Although we, as yet, have to do without an established explanation for the different distribution patterns of myotonia in ClCh and NaCh, we hypothesise that they may partly be explained by the fact that in most ClCh patients the eyelid muscles are already 'warmed-up' as a result of blinking, while a subgroup of NaCh patients still show myotonia. Alternatively or concurrently, the distribution patterns of voltage-gated chloride and sodium channels may differ for the various muscles. For example, human facial muscles probably contain more sodium channels than human skeletal muscles.[22,23]

The high incidence of the warm-up phenomenon (76.7%) in our NaCh group is remarkable. Initially, the warm-up phenomenon was predominantly reported for ClCh and was only rarely observed in NaCh.[10] However, in about 20% of our patients with the initial suspicion of a "DMC" phenotype, we detected a mutation in *SCN4A* (NaCh).[14] All these patients were sodium-channel myotonias (SCM).[14] Hence, the warm-up phenomenon is a clinical feature in RMC, DMC as well as in SCM. Although the warm-up phenomenon has now been established as a clinical feature in both types of NDMs, its pathophysiological mechanism is still unclear.[24]

Transient paresis is a unique clinical feature in ClCh. The prevalence (62.5%) of this phenomenon in our patients with *CLCN1* mutations is comparable with earlier reports of RMC.[25,26] This is congruent with the fact that only two of our patients were diagnosed with DMC. Due to the patient distribution in the Netherlands, this phenomenon cannot distinguish RMC from DMC. However, Fialho et al. showed that generalised muscle hypertrophy, transient paresis, and depressed tendon reflexes occurred more frequently in RMC than in DMC.[19]

In parallel with the transient paresis in ClCh, our study confirmed that paradoxical myotonia is unique for NaCh. However, the symptom was only observed in 30.0% of our NaCh patients, but it should be noted that we saw no patients with HYPP, only seven patients with PC and a relatively large number of patients with SCM. Furthermore, we only tested at room temperature. Future studies should also test patients at lower temperatures, although in these conditions some patients with PC will show paradoxical myotonia while others will exhibit a flaccid (periodic) paralysis.[21]

A crucial point in the literature of NDMs is the quantification of myotonia.[27] For this reason dedicated equipment and computerised protocols to quantify myotonia in hand muscles have been employed.[28,29] However, the purpose of our study was not to quantify myotonia in the hand muscles, but to investigate using daily practice neurological examination, the clinical pattern of myotonia in three different body regions (face, hands, legs) for ClCh as well as for NaCh. Moreover, the employed devices are not freely available and neither useful for facial nor for leg muscles. Therefore, we developed standardised clinical bedside tests, which are easily applicable in every outpatient clinic.[10] We were the first who developed such a standardised, robust and detailed methodology for clinical bedside tests of myotonia in three different body regions. Actually, all patients showed clear myotonia in at least one body region. It is the ultimate goal to quantify these phenomena with special devices, as the resultant parameters will be important to set clinical endpoints in future randomised controlled trials. As we were aware of the crucial points about the quantification of myotonia, we only used the presence or absence of the different clinical phenomena for our multivariate logistic regression analysis.

A formal test-retest assessment is the ultimate guarantee for the quality of our study. However, myotonia especially fluctuates under different conditions. We standardised these conditions. Furthermore, a trained clinical team pilot tested the draft standardised neuromuscular examination and also repeated this in several patients. We detected robust and reliable data. Subsequently, the clinical team amply trained one examiner who performed all clinical bedside tests. At last, we have unpublished data of the same patient population for high density surface-EMG. Hereby we also observed force profiles. Actually, all patients with a transient paresis in our study (n=20) showed a decline in their force of the left biceps shortly after contraction (data not shown). Subsequently, all 20 patients showed an increase of their force after repetitive contractions. Since none of the other patients showed such force profiles, the presence of transient paresis was confirmed by a retest. Of course we are aware that such a good test-retest result is probably not the case for the warm-up phenomenon and paradoxical myotonia. However, the standardised interviews support our data.

The aim of our study was to redefine the clinical phenotypes of ClCh and NaCh. The results allowed us to propose clinical guidelines for genetic testing (Figure 6.1). In 2006 Fournier et al. proposed electrophysiological guidelines to focus genetic testing.[30] They distinguished three different repetitive nerve stimulation patterns.[30] Since a repetitive nerve stimulation test detects hypo-excitability of the skeletal muscle membrane, clinically observed as muscle weakness, the clinical features observed in the different subgroups of NDMs may explain the different patterns detected by Fournier. For instance, most patients with ClCh experience transient paresis followed by the warm-up phenomenon. This short period of muscle weakness corresponds with the initial hypo-excitability, followed by a progressive increase of CMAP amplitude after the short exercise test by Fournier (EMG pattern II). This effect is not shown in sodium-channel myotonias (SCM). These patients do not show any kind of muscle weakness and also show a very stable repetitive nerve stimulation pattern (pattern III). The frequent warm-up phenomenon detected in these patients could be puzzling. However, the warm-up phenomenon in ClCh reduces transient paresis (muscle weakness) as well as myotonia. Since there is no muscle weakness in SCM, the warm-up phenomenon in SCM probably only reduces myotonia. It would be interesting to correlate clinical and neurophysiological tests in the SCM in more detail. At last, paramyotonia congenita (PC) patients may show either paradoxical myotonia and/or weakness after successive short exercises. We only tested paradoxical myotonia and have no clinical correlate with the progressive hypo-excitability detected by Fournier (pattern I). Most probably successive short exercises of the same muscle group may clinically induce such a weakness in this group of patients. Finally, Fournier detected a hypo- or in-excitability of the muscle membrane after cold exposure in PC patients. We detected a paralysis of the hand muscles in two of the seven PC patients after cold exposure (data not shown). However, since it was too difficult to define a standardised clinical outcome measurement after cold exposure for PC patients (PC patients after cold exposure may show muscle weakness, paradoxical myotonia or paralysis), we removed this item from our protocol. Moreover, the clinical features in PC highly depend on the type of mutation.[30] Nevertheless, standardised clinical tests after cold exposure should also be developed.

Conclusions

We systematically investigated 62 patients with a genetically confirmed diagnosis of non-dystrophic myotonic syndromes and redefined the clinical phenotypes of chloride and sodium channelopathies. The results allowed us to propose clinical guidelines for genetic testing. These may help clinicians to perform focussed genetic analysis of either *CLCN1* or *SCN4A*. However, if this test is negative, the other gene should subsequently be analysed, too.

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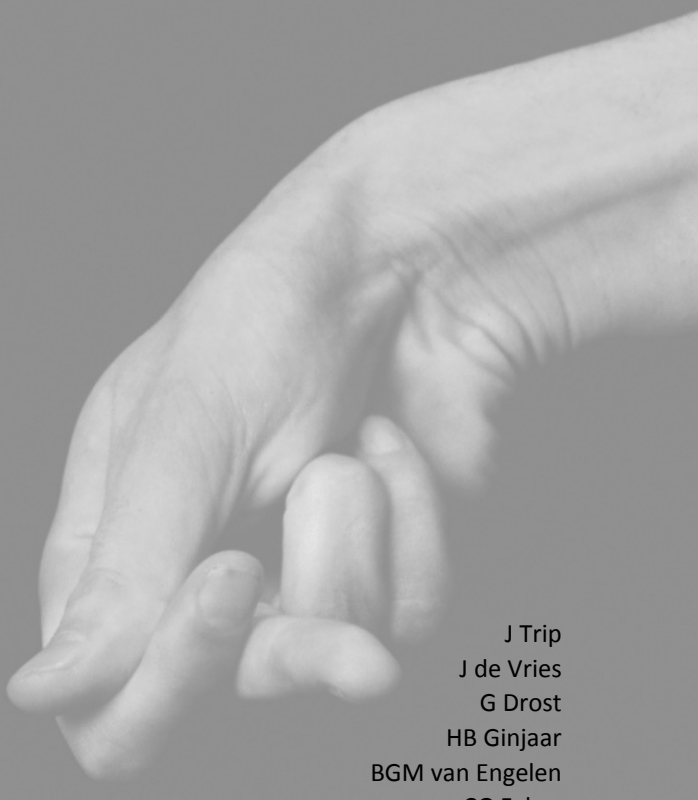
Chapter 7

Health status in non-dystrophic myotonic syndromes: close relation with pain and fatigue



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Abstract

Objective

To determine self-reported health status in non-dystrophic myotonic syndromes (NDMs) and its relationship to painful myotonia and fatigue.

Methods

In a cross-sectional study 32 NDM patients with chloride (ClCh) and 30 with sodium channelopathies (NaCh), all off treatment, completed a standardised interview, the fatigue assessment scale (FAS), and the 36-Item Short-Form Health Survey (SF-36). Beside formal assessment of pain, assessment of painful or painless myotonia was determined. The domain scores of the SF-36 were compared with Dutch community scores. Apart from the relationship among SF-36 scores and (1) painful myotonia and (2) fatigue, regression analyses in both NDM groups were conducted to determine the strongest determinants of the SF-36 domains general-health perception, physical component (PCS) and mental component summary (MCS).

Results

All physically oriented SF-36 domains in both NDM groups ($p \leq 0.01$) and social functioning in the patients with NaCh ($p=0.048$) were substantially lower relative to the Dutch community scores. The patients with painful myotonia (41.9%) scored substantially ($p<0.05$) lower on most SF-36 domains than the patients without painful myotonia (58.1%). Fatigued patients (53.2%) scored substantially lower ($p \leq 0.01$) on all SF-36 domains than their non-fatigued counterparts (46.8%). The regression analysis showed that fatigue was the strongest predictor for the general-health perception and painful myotonia for the physical component summary. None of the patients showed below-norm scores on the domain mental component summary.

Conclusion

The impact of NDMs on the physical domains of patients' health status is substantial, and particularly painful myotonia and fatigue tend to impede their physical functioning.

Introduction

Non-dystrophic myotonic syndromes (NDMs) are caused by mutations in genes encoding the skeletal muscle chloride (*CLCN1*) or sodium channel (*SCN4A*). Mutations in *CLCN1* are responsible for recessive and dominant myotonia congenita (recessive MC [MIM 160800] and dominant MC [255700]), and mutations in *SCN4A* for paramyotonia congenita (PC [MIM 168300]) and potassium-aggravated myotonias (PAM [MIM 608390]).[1]

The key symptom of MC is myotonia, and especially patients with recessive MC show a combination with transient paresis.[2] Both symptoms improve with continuing exercise, which is referred to as the warm-up phenomenon.[3,4] Conversely, in PC myotonia worsens with continuing exercise. Muscle weakness is also observed in PC and is mostly elicited by cold or prolonged exercise.[5] Myotonia in PAM is clinically characterised by unusual features such as temporal fluctuation (myotonia fluctuans), permanent myotonia (myotonia permanens) or acetazolamide-responsive myotonia (acetazolamide-responsive myotonia congenita).[6-8]

Myotonia in NDMs is often described as painless muscle stiffness.[9] However, Becker found 15% of his patients with recessive MC to report pain complaints.[2] Also, a fluctuating form of dominant MC, acetazolamide-responsive myotonia congenita and the V445M sodium channel mutation were associated with painful myotonia; thus, pain appears to be a recurrent symptom in NDMs.[2,8,10,11]

Another symptom in neuromuscular diseases is fatigue. Although initially under-reported in neuromuscular disorders, fatigue has now been established in post-polio syndrome, immune-mediated polyneuropathies, myasthenia gravis, facioscapulo-humeral muscular dystrophy, myotonic dystrophy, and type-I hereditary motor and sensory neuropathy.[12-16] Our clinical experience has taught us that also patients with NDMs may complain of fatigue.

Despite their symptoms and signs, NDMs are considered to be a group of 'benign diseases' with possible influences on the patients' ability to perform activities of daily living, affecting their social participation.[17] This explains why to date no clinical reports are available that have systematically examined the effects NDMs have on the patients' health status, where health status is defined as the impact of the disease on a patient's physical, psychological and social functioning.[18] The presence of painful myotonia and fatigue in patients with NDMs and their impact on the patients' health status has likewise not been systematically investigated. Therefore, we investigated the impact of NDMs on health status. We additionally examined the presence and effects of painful myotonia and fatigue and, finally, performed regression analyses to

investigate the contribution of various determinants assumed to impair the patients' physical, psychological and social functioning.

Patients and methods

Patients

In March 2005 neurologists across The Netherlands as well as the Dutch Patient Association for Neuromuscular Diseases (Vereniging Spierziekten Nederland) were requested to report all patients with a clinical suspicion of NDMs to our research group for a full year. Inclusion criteria were age (≥18 years), a diagnosis of NDM according to established clinical criteria and needle-EMG evidence of myotonic discharges.[19] Exclusion criteria were a clinical or genetic diagnosis of myotonic dystrophy type 1 or 2, primary periodic paralysis, serious co-morbidity, unwillingness or inability to reduce or stop drug therapy for myotonia during the study and no detectable mutations in *CLCN1* or *SCN4A*.

The 1-year recruitment period yielded a total of 97 potentially eligible patients. Nine patients were unable to participate due to transportation problems. Based on the selection criteria, another 26 patients were excluded: 7 had a primary periodic paralysis, 12 serious co-morbidity, 5 were unwilling to reduce and stop myotonia-related drug therapy, and 2 did not present clinical signs of myotonia and lacked myotonic discharges (needle-EMG). All remaining 62 patients demonstrated DNA mutations in either *CLCN1* (n=32) or *SCN4A* (n=30).[20,21] The study was approved by the Medical Ethics Committee of the Radboud University Nijmegen Medical Centre, and all patients gave their written informed consent prior to their participation.

Examination procedures

Standardised interview

With the standardised interview the following characteristics of all NDM patients were established: age, gender, duration of symptoms, frequency, pattern (reduction or augmentation of myotonia after repetitive movements) and severity of the myotonia [Numerical Rating Scale (NRS), range 1-10; 1 means almost no myotonia and 10 means very serious myotonia], changes in severity, presence of muscle weakness, presence of painful or painless myotonia and eventually the severity of the painful myotonia (NRS, range 1-10; 1 means almost no painful myotonia and 10 means very serious painful myotonia).

Painful myotonia

We used the outcome of the standardised interview to dichotomise patients with painless and those with painful myotonia. Patients with painful myotonia were also asked about the severity of their pain (NRS, range 1-10).

Fatigue assessment scale

Fatigue was assessed with the ten-item self-reported fatigue assessment scale (FAS).[22] Five items probe physical and five mental fatigue. Each item is scored on a 5-point Likert scale with the total score ranging from 10 to 50. Higher scores indicate higher fatigue levels. The scale's validity and reliability of the FAS were good.[22-24] We took FAS scores equal to or higher than 22 to be indicative of fatigue and used this as the cut-off score to dichotomise fatigued and non-fatigued NDM patients.[24]

Short-Form 36-Item Health Survey

Patients completed the Short-form 36-Item Health Status Survey (SF-36, Dutch version), a generic questionnaire to establish patients' self-reported health status.[25,26] It appraises the following domains: physical functioning (10 items), role functioning physical (4), role functioning emotional (3), social functioning (2), body pain (2), mental health (5), vitality (4), general-health perception (5), and change in health, which is scored separately.[26] The number of response categories per item ranges from two to six. Each domain has a scoring range of 0-100 with higher scores indicating better health or functioning and less body pain. The scale's physical component summary (PCS) and mental component summary (MCS) scores were also calculated using the reported means, standard deviations and factor-score coefficients derived from the general Dutch population.[25-28] The PCS captures the individual's overall physical functioning by considering reported limitations in care, physical, social and role activities, amount of pain, and level of energy. The MCS reflects the respondent's overall mental functioning by considering the frequency of psychological distress and limitations in usual social and role activities due to emotional problems.[27] A linear T score transformation method was used so that the PCS had a mean of 49.7 (SD 9.3) and the MCS had a mean of 52.1 (SD 9.6), as reported for the general Dutch population.[25-28] The SF-36 has been shown to have a good validity and reliability.[25-28] For our comparative analyses, we used the reported mean (SD) SF-36 domain scores of a random, Dutch nationwide sample of 1,742 healthy individuals consisting of 976 men and 766 women with a mean age of 47.6 years (18.0 years; range 16-94 years).[25,28]

Statistical analysis

Validity and reliability

Prior to the health status analyses, we tested the validity and reliability of the scales used. Validity was determined by means of Spearman's rank correlation tests among (1) the various SF-36 dimensions, (2) the SF-36 dimension vitality and FAS and (3) the SF-36 domain body pain and painful myotonia. The reliability of the SF-36 and FAS was estimated by calculating the internal consistency (Cronbach's α). A Cronbach's $\alpha \geq 0.7$ is considered as having a good reliability.[29]

SF-36 values

The mean SF-36 domain and summary (PCS and MCS) values for (1) chloride (ClCh) and sodium channelopathies (NaCh), (2) the fatigued and the non-fatigued group and (3) the painful and painless myotonia group were compared with Dutch community scores (Student's *t* test for independent groups).[25,28] In addition, the SF-36 domain scores of the patients with a ClCh were compared with those with a NaCh, the fatigued patients were compared with non-fatigued patients, and those patients with painful myotonia were compared with their painless counterparts (Student's *t* test for independent groups). [25, 28]

Regression analysis

In both NDM groups linear regression analyses were performed to determine which variable had the greatest impact on the patients' general-health perception, PCS and MCS (dependent variables). Age, gender, duration of symptoms, frequency of myotonia, its pattern after repetitive contractions, myotonia severity and changes in severity, presence of muscle weakness, presence of painful myotonia, severity of the pain and the FAS were considered as explanatory variables. Prior to the regression analyses, the distribution pattern of the dependent variables were examined and, if necessary, a transformation of the values was carried out to obtain a normal distribution pattern. Univariate regression analyses were conducted to reveal those independent variables that were related to dependent variables, separately. This was achieved through a systematic evaluation of constructed graphs with linear regression studies that included a restricted cubic spline function on the independent variable.[30] Subsequently, separate multivariate linear regression analyses were performed for each dependent variable, using the stepwise method including only the independent variables that had shown $p \leq 0.2$ in the univariate analyses. The strength of the association between the dependent variable and the independent variables was presented as R^2: the fraction of variance explained by the independent variables from the regression model. All analyses were performed using Stata 7.0 for Windows 2000 (Stata Corp., College Station, TX). A value of $p<0.05$ was considered statistically significant.

Results

Interview outcomes

Table 7.1 shows the basic characteristics of all NDM patients obtained during the standardised interview. All patients complained of myotonia, and the majority (96.9% in ClCh and 83.3% in NaCh) reported daily complaints. All the patients with ClCh reported a decrease in myotonia after repetitive contractions, which was 50% for the patients with a NaCh, while the other half indicated an increase (paradoxical myotonia). Although ten patients in the chloride group and eight patients in the sodium group used drugs to relieve myotonia before the study, both groups claimed the severity of their myotonia had increased since the onset of symptoms. Over half of all patients with NaCh (56.7%) and about 30% (28.1%) of the patients with ClCh characterised their myotonia as painful (χ^2=5.18, p=0.02). Finally, 75% of the ClCh versus 37% in the NaCh experienced muscle weakness (χ^2=9.26, p=0.002).

Table 7.1 Basic characteristics of all patients with a non-dystrophic myotonic syndrome (NDM), for each subtype separately.

	ClCh (n=32)	**NaCh (n=30)**	**p-value**
Gender, n (%)			0.131
Men	20 (62.5)	13 (43.3)	
Women	12 (37.5)	17 (56.7)	
Mean age in years (SD), range	45.7 (10.6), 23-60	38.7 (12.3), 19-68	0.019 *
Mean duration of symptoms (SD), range	36.1 (12.8), 9-57	34.3 (13.5), 6-68	0.599
Frequency of myotonic symptoms, n (%)			0.184
On a daily basis	31 (96.9)	25 (83.3)	
Several times a week	1 (3.1)	4 (13.3)	
Several times a year	0 (0)	1 (3.3)	
Myotonia after repetitive movements, n (%)			<0.001 *
Reduced (warm-up phenomenon)	32 (100)	15 (50.0)	
Increased (paramyotonia)	0 (0)	15 (50.0)	
Severity of myotonia			0.459
(1 = very mild - 10 = most severe), n (%)			
1-5	13 (40.6)	15 (50.0)	
6-10	19 (59.4)	15 (50.0)	
Changes in severity of myotonia, n (%)			0.541
Increasing	19 (59.4)	17 (56.7)	
Stable	12 (37.7)	10 (33.3)	
Decreasing	1 (3.1)	3 (10.0)	
Muscle weakness, n (%)			0.002 *
Yes	24 (75.0)	11 (36.7)	
No	8 (25.0)	19 (63.3)	
Painful versus painless myotonia, n (%)			0.023 *
Painful myotonia	9 (28.1)	17 (56.7)	
Painless myotonia	23 (71.9)	13 (43.3)	
Severity of painful myotonia			0.443
(1 = very mild - 10 = most severe, n (%)			
0	23 (71.9)	13 (43.3)	
1-5	4 (12.5)	5 (16.7)	
6-10	5 (15.6)	12 (40.0)	

* p<0.05

Incidence painful myotonia

Twenty-six (41.9%) patients reported painful myotonia with a mean NRS score of 6.1; 36 patients (58.1%) reported painless myotonia. There was neither a relationship between the presence of painful myotonia and gender (χ^2=0.287, p=0.670), nor between the presence of painful myotonia and age (r=- 0.07, p=0.60).

Fatigue rates by FAS

Based on their FAS scores, 33 (53.2%) patients were classified as fatigued (FAS 27.2±4.3) and 29 (46.8%) as non-fatigued (FAS 16.2±3.5). In ClCh 15 patients were fatigued (46%) and in NaCh 18 patients were fatigued (60%), (χ^2=1.07, p=0.30). The FAS scores showed neither a relationship with gender (r=0.10, p=0.440), nor with age (r=- 0.05, p=0.70).

FAS and SF-36 validity and reliability scores

The matrix in Table 7.2 shows correlations among (1) the various SF-36 domains, (2) the SF-36 domain vitality and the FAS and (3) the SF-36 domain body pain and painful myotonia for the two NDM groups separately. For most SF-36 dimensions significant correlations were obtained. In both groups the SF-36 scores for vitality (higher scores mean better vitality) correlated inversely with the FAS scores (higher scores mean more fatigued), and the SF-36 scores for body pain (higher scores mean less pain) correlated inversely with painful myotonia (higher scores mean more pain). Table 7.2 also shows the internal consistencies for the SF-36. For all SF-36 dimensions, good reliability scores (Cronbach's alpha ≥0.7) were obtained. Cronbach's alpha for the FAS was also good: 0.81 for ClCh and 0.90 for NaCh.

SF-36 outcomes

Chloride and sodium channelopathies

Compared with the Dutch community scores, the means for the physically oriented SF-36 domains (physical functioning, role functioning physical, vitality and general-health perception) and the corresponding PCS means were notably lower (p≤0.01) in both NDM groups, indicative of an inferior health status in the patients (Figure 7.1a). The means for the more mentally oriented SF-36 domains (role functioning emotional, social functioning, mental health and body pain) and MCS were comparable with Dutch community scores, with social functioning in the patients with NaCh (p=0.048) as the only exception. The body pain scores in the chloride group were even significantly higher (indicating less pain) than in the Dutch community (p=0.014). The SF-36 domain scores and the component scores did not differ between the two NDM groups, with the exception of the body pain scores, which were significantly lower (indicating more pain) in the sodium group (p=0.032).

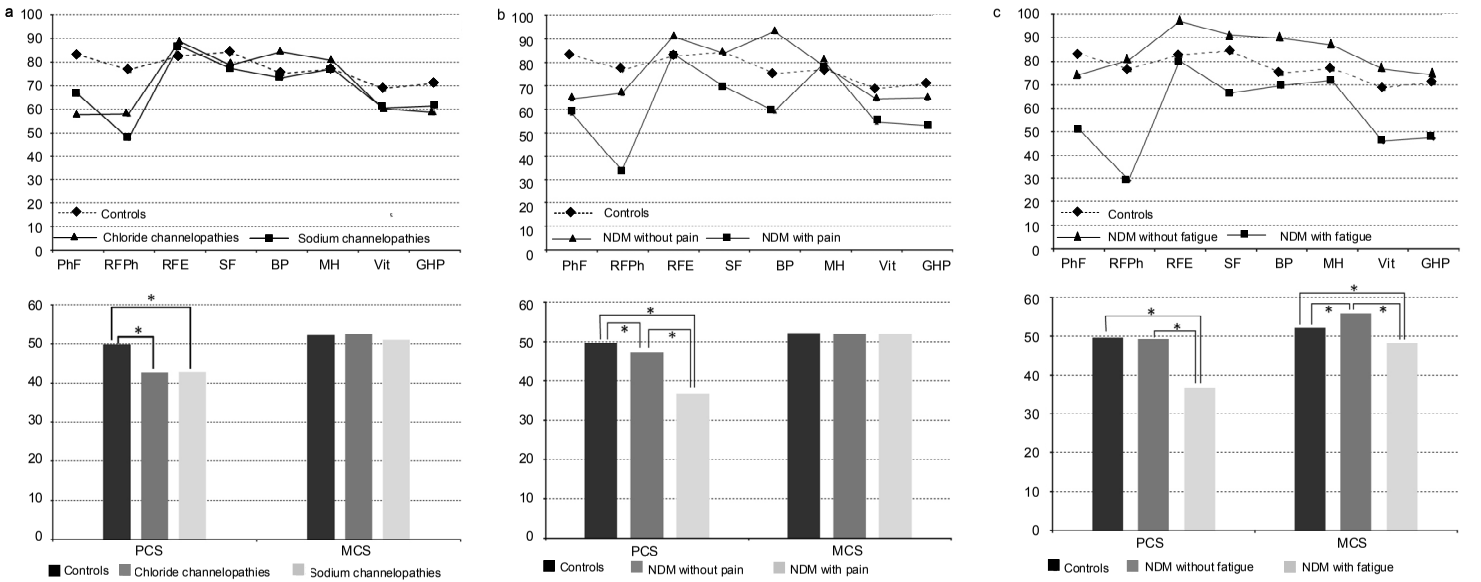

Figure 7.1 (a) A comparison of the mean values of the 36-item Short-Form health survey profiles (SF-36) from patients with NDM (n=62) per subtype and the mean values for the Dutch community. (b) The effect of painful myotonia on the patients' mean scores on the SF-36 versus the reported means for the Dutch community. (c) The effect of fatigue on the patients' mean scores of the SF-36 scores versus the reported mean for the Dutch community.
NDM: non-dystrophic myotonic syndrome. PhF: physical functioning; RFPh: role functioning physical; RFE: role functioning emotional; SF: social functioning; BP: body pain; MH: mental health; Vit: vitality; GHP: general-health perception; PCS: physical component summary; MCS: mental component summary.
* $p<0.05$.

Table 7.2 Content validity of the SF-36, the SF-36 domain vitality and the FAS and the SF-36 domain body pain and painful myotonia for the two NDM groups separately. It also shows the internal reliability of all SF-36 domains.

	PhF	RFPh	RFE	SF	BP	MH	Vit	GHP	PCS	MCS
Validity studies (Spearman's rank correlation coefficients)										
PhF	Chl / Sod									
RFPh	0.73 / 0.68	Chl / Sod								
RFE	NS / 0.43	0.42 / 0.55	Chl / Sod							
SF	0.38 / 0.39	0.43 / 0.56	NS / 0.57	Chl / Sod						
BP	0.38 / 0.49	0.50 / 0.59	NS / 0.31	NS / 0.59	Chl / Sod					
MH	NS / 0.56	NS / 0.67	0.40 / 0.52	0.54 / NS	NS / 0.33	Chl / Sod				
Vit	0.36 / 0.53	0.40 / 0.65	NS / 0.42	0.52 / 0.63	0.31 / 0.63	0.47 / 0.59	Chl / Sod			
GHP	0.31 / 0.50	0.46 / 0.67	NS / NS	NS / 0.65	0.38 / 0.80	NS / 0.40	0.52 / 0.76	Chl / Sod		
									Chl / Sod	Chl / Sod
FAS							-0.85 / -0.90			
Painful myotonia					-0.76 / -0.67					
Internal consistency (Cronbach's alpha)										
	0.87 / 0.94	0.85 / 0.94	0.81 / 0.84	0.79 / 0.80	0.77 / 0.88	0.81 / 0.75	0.82 / 0.79	0.71 / 0.87	0.79 / 0.87	0.86 / 0.87

NDM: non-dystrophic myotonic syndrome, PhF: physical functioning, RFPh: role functioning physical, RFE: role functioning emotional, SF: social functioning, BP: body pain, MH: mental health, Vit: vitality, GHP: general-health perception, PCS: physical component summary, MCS: mental component summary, FAS: fatigue assessment scale. Correlations were significant ($p<0.05$), except NS (not significant).

Painful and painless myotonia

Painful myotonia was associated with a significant decrease ($p \leq 0.0001$) of all SF-36 domain scores relative to the Dutch community scores, except for the domains role functioning emotional, mental health and MCS (Figure 7.1b). The values for role functioning emotional, social functioning, body pain and mental health for the patients that not report painful myotonia were equal to or higher than the Dutch community scores, whereas their means for physical functioning, general-health perception and PCS were significantly ($p \leq 0.048$) lower. The pain group showed significantly lower ($p \leq 0.034$) mean scores than the non-pain group for role functioning physical, social functioning, body pain, vitality, general-health perception and PCS.

Fatigued and non-fatigued patients

All SF-36 domain scores of the fatigued patients were significantly lower ($p \leq 0.01$) than the Dutch community scores, except for the domains role functioning emotional, mental health and body pain (Figure 7.1c). The means for the non-fatigued patients were equal or higher than the Dutch community scores, apart from the mean for physical functioning ($p=0.02$). All SF-36 domain and summary scores of the fatigued patients were substantially lower ($p \leq 0.01$) than those found than their non-fatigued counterparts (Figure 7.1c).

Regression analysis

As the MCS scores (reflecting overall mental functioning) for our NDM patients closely resembled the Dutch community scores, we restricted our regression analyses to general-health perception and PCS (see Figure 7.1a). The results of the analyses are shown in Figure 7.2. In the chloride group, 46% of the general-health perception scores and 46% of the PCS scores were explained by the explanatory variables. The variance proportions obtained in the sodium group were higher: 77% for general-health perception and 71% for PCS. Fatigue proved the strongest predictor of the general-health perception ($p \leq 0.01$ for ClCh and $p \leq 0.0001$ for NaCh). In the sodium group the pattern of myotonia ($p=0.001$), gender ($p=0.002$), painful myotonia ($p=0.009$), and duration of symptoms ($p=0.019$) also significantly contributed to the model. Painful myotonia was the strongest contributor to the PCS score in the ClCh ($p=0.001$), in whom fatigue was also a significant ($p=0.045$) contributor. In NaCh, painful myotonia ($p=0.018$), fatigue ($p=0.018$), and pattern of myotonia ($p=0.023$) were also significant contributors to the PCS score.

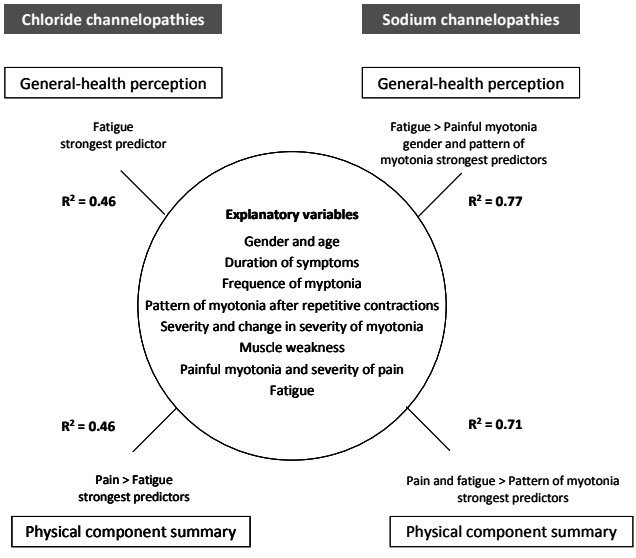

Figure 7.2 Overview of the outcomes of the multivariate regression analyses conducted to establish the variance and strongest predictor of the general-health perceptions and the physical component summary in patients with NDMs.

Discussion

Despite the general notion that NDMs are benign diseases, the current cross-sectional study demonstrates that the symptoms of these patients do greatly impact their self-reported health status. To our knowledge, ours is the first study to investigate health status in a large group of genetically confirmed NDM patients. The scores of the NDM patients on the scale's physically oriented domains were substantially lower than the scores reported for the Dutch community (Figure 7.1a), reflecting a poorer clinical condition for the patients.[25-28] In addition, 42% of the patients characterised their myotonia as painful and, based on their FAS scores, 53% could be classified as being fatigued. Furthermore, both painful myotonia and fatigue were associated with a significant decrease in almost all SF-36 domain scores (Figure 7.1b and 7.1c). Finally, our regression analyses revealed that in our sample fatigue was the strongest predictor of low general-health perceptions, while painful myotonia best predicted low overall physical functioning (Figure 7.2).

The relatively low scores we found in our NDM patients for the physical domains of the SF-36 closely resemble the scores of other chronic diseases like type-II diabetes mellitus, myocardial infarction and angina pectoris with hypertension, as reported in the USA population by the constructors of the SF-36.[39] In contrast, most of the NDM

patients' scores on the scale's mental domains and its mental component score were quite similar to the Dutch community scores.[25-28] Studies of elderly patients with chronic conditions, including various peripheral neuropathies and eight chronic medical disorders, also found lower scores on the SF-36 physical dimensions and an unaltered mental state.[14,31,32] Presumably, these patients are preoccupied by changes in their physical status, making them more focused on physical disabling symptoms. Since NDMs are chronic and presumably indolent, progressive diseases, it is possible that patients might learn to cope with their limitations. Indirect support for this hypothesis is found in studies of more pronounced progressive chronic illness, such as myotonic dystrophy, ALS, Parkinson disease and multiple sclerosis. Here, the SF-36 showed the patients' emotional and mental state to be more affected, although involvement of the central nervous system could not be excluded.[33-37]

The physical domains of the SF-36 from NDM patients with painful myotonia were substantially more affected than those from patients with painless myotonia. Pain had earlier been observed to correlate with lower scores on physical domains of the SF-36 in other slowly progressive neuromuscular diseases.[38] Fatigue resulted in our study even in a substantial decrease of all SF-36 domains. A negative impact of fatigue on health status was also reported earlier in some chronic neurological diseases and various muscular dystrophies.[13,39,40] On the other hand, NDM patients without pain or fatigue often had similar or even higher SF-36 scores (better health condition) than the normal Dutch population. This confirms earlier assumptions that there indeed is a group of NDM patients with only a minor or no impact on their health status.

Since Aaronson et al.[25] studied the SF-36 domain scores in different age classes of the Dutch population and detected lower scores with increasing age, age differences among the Dutch population, the NDM population and the NDM subgroups should be discussed in the evaluation of the results. However, since our data showed no correlation between age and the domains of the SF-36 (data not shown), including the physical component summary and the general-health perception, age differences do not appear to play a role. Furthermore, the mean age of the total NDM population (42.3±11.9 years) is even lower than the mean age of the Dutch population studied by Aaronson (47.6±18.0 years).[25] Therefore, the lower SF-36 scores of our NDM population are most probably not caused by age differences. Also, the mean age of the two patient subgroups (painful myotonia versus painless myotonia and fatigued versus non-fatigued) showed no significant differences. Only the NDM subgroup ClCh versus NaCh showed a significant difference in age: ClCh were significantly older (Table 7.1). This is the only group in which we could not pertinently rule out a influence of age differences.

As explained in the introduction, NDMs are not a single disease. However, in this manuscript we only compared ClCh with NaCh. Of course, for more thorough genotype-phenotype correlation studies, a further subdivision should be made. Since the goal of this manuscript was not to describe the different diseases as such but the burden of the total NDM group in daily life and the fact that we have only seen small groups for the different diseases, which makes the groups possibly too small for statistical analysis, we did not analyse more detailed genotype-phenotype correlations.

Some permanent muscle weakness may be a feature of NDMs, especially in recessive myotonia congenita and some NaCh. This could be a confounder. However, since most patients with recessive myotonia congenita experience transient paresis, chronic muscle weakness is very difficult to measure in this group. In the NaCh all patients showed a maximum MRC sum score (data not presented). Furthermore, none of the patients showed such severe myotonia that muscles became functionally weak. We therefore only used standardised interview parameters in the regression analyses. We used different variables, including frequency of myotonia, severity of myotonia, and muscle weakness, as explanatory variables. None of these, in contrast to fatigue and pain, were a significant variable for the variance of the general-health perception or the physical component summary.

Prior to this study, no valid and reliable health status measures had been obtained in patients with NDMs. We opted for the SF-36 because of its brevity, its extensive use in clinical studies and its good psychometric properties.[25-28] We selected the FAS to measure fatigue because of its one-dimensionality, ease of use and proven validity and relability.[22-24] The scales' good content validity and reliability make them potentially useful endpoints for future randomised clinical trials that evaluate the efficacy of and responsiveness to drug treatments such as mexiletine.

Despite the solidity of our nationwide findings, it needs to be noted that we did not evaluate the test-retest reliability for the SF-36 and FAS, a psychometric omission future research projects in this field should correct. Furthermore, five patients were unwilling to reduce and stop their drug therapy, and nine patients had transportation problems, including one patient who was severely handicapped. This means that there is a potential bias in this study. Possibly some patients with more severe myotonia have not been assessed. However, these patients may even have a worse health status than the rest.

In summary, the current study shows that NDMs substantially affect the patients' physical health status, with painful myotonia and fatigue being the strongest predictors of the deficits. Beside direct measurements of myotonia, it is recommended that future intervention studies in patients with NDMs should also use the SF-36 and FAS to establish the efficacy of the therapeutic regimes under study.

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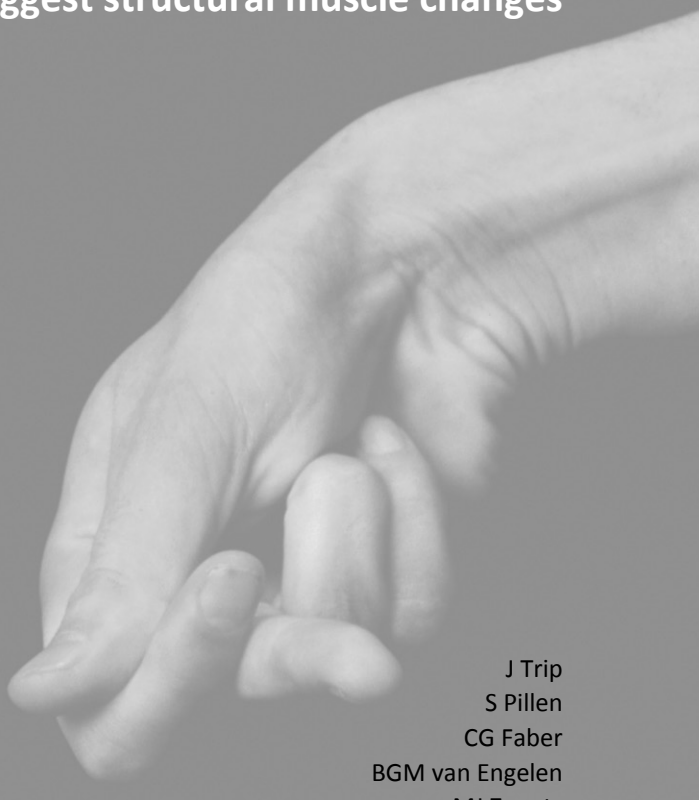
Chapter 8

Muscle ultrasound measurements and functional muscle parameters in non-dystrophic myotonic syndromes suggest structural muscle changes



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Abstract

Background
Patients with non-dystrophic myotonic syndromes, including chloride (myotonia congenita) and sodium channelopathies (paramyotonia congenita and potassium aggravated myotonias), may show muscular hypertrophy in combination with some histopathological abnormalities. However, the extent of muscle changes has never been objectified in a large group genetically confirmed patients.

Objective
To determine echo intensities, echo thickness, ranges of motion and force of four skeletal muscles in genetically confirmed patients with NDM.

Methods
In 63 genetically confirmed patients with NDM we examined four skeletal muscles in the transverse plain by ultrasound: left biceps brachii, right forearm flexors, right quadriceps femoris, and left tibialis anterior muscle. Mean muscle echo intensity was quantified using a computer-assisted grey-scale analysis. In each muscle a predefined region of interest in the ultrasound image was selected and the mean grey value was calculated. Muscle thickness was measured with electronic callipers at predefined standardised locations in the ultrasound image.
Active ranges of motion of the left elbow, right wrist, right knee and left ankle were measured in degrees using a transparent goniometer with standardised positions for patients, examiner and goniometer. Using the 'make' technique, the maximum isometric contraction values of the left elbow flexion, right three-point grip, right knee extension and left ankle dorsiflexion were measured with a calibrated hand-held dynamometer.

Results
The main findings revealed elevated echo intensities in all muscles except the rectus femoris (+ 1.3-2.2 SD, $p<0.0001$), and hypertrophy of the arm muscles (+ 0.5-0.9 SD, $p<0.01$). The echo intensities of the dominant biceps brachii muscle and the dominant forearm flexor muscles were significantly higher ($p=0.002$ and $p=0.038$, respectively) than the levels observed for the non-dominant side.
Muscle echo intensities were inversely correlated to the corresponding ranges of motion (biceps brachii: $r=-0.43$; $p<0.001$, forearm flexors: $r=-0.47$; $p<0.001$, rectus femoris: $r=-0.40$; $p=0.001$, and tibial anterior: $r=-0.27$; $p=0.04$) and positively correlated to age ($r=0.22$; $p=0.05$). The echo intensity of the forearm flexors was inversely correlated to their muscles' force ($r=-0.30$; $p=0.02$).

Conclusion
These data suggest that non-dystrophic myotonic syndromes may lead to structural muscle changes that increase with age and muscle use.

Introduction

Non-dystrophic myotonic syndromes (NDMs) form a group of rare hereditary diseases, existing of either chloride (recessive and dominant myotonia congenita) or sodium channelopaties (paramyotonia congenita/potassium aggravated myotonias), that exclusively affect skeletal muscles.[1-3] The key symptom of NDMs is myotonia, i.e. a delayed muscle relaxation after a voluntary or evoked contraction. Needle-electromyography of patients with NDMs show myotonic discharges in almost all skeletal muscles, even at rest.[4,5] Both myotonia and myotonic discharges are the consequence of a hyperexcitable sarcolemma caused by a disturbed chloride or sodium conductance. Recently, the molecular basis of NDMs was discovered allowing the diagnosis to be confirmed by genetic testing.[6,7]

The term NDMs by definition implies that, in contrast to the dystrophic myotonic syndromes (myotonic dystrophy type 1 and type 2), they neither show dystrophy, nor structural muscle changes. Indeed, muscle biopsies of NDMs were initially described as normal or showing mild, non-specific abnormalities.[8] Subsequent investigations reported an absence of type 2B fibres in chloride channelopathies (ClCh), while patients with paramyotonia congenita, a sodium channelopathy (NaCh), were found to show intracellular large vacuoles and tubular aggregates.[9-11] However, the presence of muscle changes has never been systematically investigated in a large group of genetically confirmed patients.

Clinically, muscle hypertrophy may be prominent in patients with NDMs and is thought to be caused by repetitive and prolonged muscle contractions. Becker reported muscular hypertrophy in 71% of his patients with recessive myotonia congenita and in 25% of the patients with dominant myotonia congenita (both ClCh).[8] In paramyotonia congenita muscular hypertrophy is reported to be rare (NaCh).[1] Even so, these findings stem from the pre-genetic era and were all solely based on subjective, visual examinations.

Muscular ultrasound has been shown to be accurate in detecting the presence and extent of muscle changes and muscle thickness.[12-16] Fibrosis and fatty infiltration as for example detected in abundance in muscular dystrophies are thought to account for an increased echo signal detected by muscle ultrasound.[12,14,17,18] Although muscle ultrasound cannot determine the exact nature of changes, this technique is non-invasive and allows easy examination of multiple muscles, yielding a clear overview of the extent and distribution of the pathology within the patient's body.[19]

In order to determine whether and where in NDMs muscle changes and muscular hypertrophy occur, we quantitatively measured the echo intensities and thicknesses of two proximal and two distal skeletal limb muscles in a large group of genetically confirmed NDM patients. We, moreover, compared the outcomes for the ClCh and NaCh and those of the male and female patients and, lastly, investigated whether the echo data were correlated to the corresponding ranges of motion, muscle force and age.

Patients and methods

Subjects

Between April 2005 and March 2006 a large cohort of NDM patients residing in the Netherlands participated in our comprehensive non-dystrophic myotonic syndrome study, 63 of whom took part in the present investigation. Inclusion criteria were a minimum age of 18 years, a clinically and genetically confirmed diagnosis of NDM according to established clinical criteria, and needle electromyographic evidence of myotonic discharges.[20] We distinguished ClCh from NaCh and paramyotonia from other NaCh (potassium aggravated myotonias or sodium-channel myotonias) by clinical criteria and DNA-analysis.[20,21] The study was approved by the local medical ethics committee and all patients gave their written informed consent prior to their participation.

Ultrasound measurements

The method of ultrasound scanning is described in detail in earlier reports.[22,23] In short, we examined four muscles in the transverse plane: left biceps brachii, right forearm flexors, right quadriceps femoris, and left tibialis anterior muscle. The apparatus used was a phased-array real-time scanner (Sonos 2000 Phased Array Imaging System; Hewlett-Packard Company, Andover, Massachusetts, USA), with a 7.5 MHz transducer. To enable us to use previously established normal values, all system parameters were equivalent to the ones we used in our previous study.[22] Mean muscle echo intensity was quantified using a computer-assisted grey-scale analysis. In each muscle a region of interest was selected according to our previous protocol and the mean grey value was calculated: 8-bit scale; black = 0; white = 255.[22] These values are related to the amount of decibels that return to the transducer, which is incorporated in the hard- and software of the ultrasound device. A predefined look-up-table in the ultrasound machine converts the decibels to corresponding grey values. In our study (as in all studies on muscle ultrasound) this look-up-table was the same in every measurement, as a fixed preset (gain and compression) was used.[22] As in severe neuromuscular disorders the outline of the vastus intermedius muscle may be difficult to delineate, we opted to use the rectus femoris in the quadriceps femoris muscle for our echo-intensity analysis.[24] Muscle thickness was measured with electronic callipers at predefined standardised locations in the ultrasound image.[22] To gauge differences in the dominant and non-dominant arm muscles we performed bilateral measurements in 38 patients (18 chloride channelopathies and 20 sodium channelopathies), with dominance having been established during the intake interview. All images were also independently evaluated visually by two investigators (JT and SP) to investigate if specific patterns of distribution or increased echo intensities were present either among different

muscles or within the muscle, as has previously been described in certain congenital myopathies or anterior horn cell disorders.[25]

Range of motion and muscle-force measurements

Active ranges of motion of the left elbow, right wrist, right knee and left ankle were measured in degrees using a transparent goniometer while adhering to Norkin and White's system with standardised positions for patients, examiner and goniometer.[26]
The maximum isometric contraction values of the left elbow flexion, right three-point grip, right knee extension and left ankle dorsiflexion were measured with a calibrated hand-held dynamometer (type CT 2001, C.I.T. Technics, Haren, the Netherlands).[27,28]
We used the 'make technique': the patients were instructed to take 1 or 2 seconds to reach maximum effort levels and subsequently contract their muscle as forcefully as possible. All measurements were conducted using standardised positions for patients, examiner and dynamometer.[28] All patients were tested in a temperature-controlled room (20°C) at the same time of day and by the same investigator (JT) to reduce variations in testing.

Data analysis

The patients' ultrasound results were compared to previously established normal values and transformed into z-scores (corrected for age and gender), i.e. the number of standard deviations above or below normal.[22,23,29] In general, z-scores are constructed in such a way that in a healthy or reference population they lead to z-scores with an average of 0 and a standard deviation of 1. This 'normalisation' helps to interpret the outcome of a variable in a population that may otherwise be difficult to interpret (or changes with age). When the z-score in a diseased population is 0, the outcome is 'normal', while values above 2 and under -2 are 'abnormal' in the sense that they occur in less than 5% of the healthy population.
Statistical analyses were performed using the SPSS package for Windows, version 11.0 (SPSS Inc, Chicago, IL, USA). A one-sample *t*-test was used to assess whether the patients showed a significant increase in their echo-intensity and muscle-thickness z-scores and an independent-sample *t*-test to compare the respective z-scores for the ClCh and NaCh and male and female patients. We ran a paired-sample *t*-test to compare the absolute values of the echo intensities and thicknesses of the dominant and non-dominant arm muscles. Correlations between muscle echo intensities and the corresponding ranges of motion and muscle forces were calculated using Pearson's correlation coefficients, as were the correlations between muscle thickness and muscle force. Echo intensities and thicknesses were correlated to age for each muscle separately as well as for their sum scores. A p-value of less than 0.05 was considered to be significant.

Results

Table 8.1 shows the primary diseases and antropometric data of all 63 participating patients. In three patients the measurements for one muscle were excluded from further analysis: the left biceps muscle in one recessive chloride channelopathy because of weakness due to a mechanical accident; the left tibialis anterior muscle in a sodium channelopathy because of chronic muscle weakness due to a previous lumbar disc hernia; the right rectus femoris muscle in a chloride channelopathy because the echo intensity could not be measured reliably as the rectus femoris was located at a depth of more than 3.5 cm and thus outside the maximum setting stipulated in the ultrasound protocol. Furthermore, knee extension appeared to exceed the investigator's force in a majority of patients and could therefore not be measured reliably.

Table 8.1 The primary genetically confirmed diseases and antropometric data of the 63 participating patients with a non-dystrophic myotonic syndrome.

Primary disease	**No of patients**	**Sex M/F**	**Age (yr) median (range)**	**Weight (kg) median (range)**	**Length (m) median (range)**
Chloride channelopathies					
Recessive myotonia congenita	31	21/10	45 (23-61)	75 (59-136)	1.72 (1.56-1.87)
Dominant myotonia congenita	3	2/ 1	60 (32-61)	89 (57- 92)	1.83 (1.57-1.89)
Sodium channelopathies					
Paramyotonia congenita	10	6/ 4	42 (23-72)	77 (45-115)	1.80 (1.65-1.96)
Sodium-channel myotonias	19	8/11	40 (20-68)	75 (51- 93)	1.72 (1.60-1.96)
Total	**63**	**37/26**	**43 (20-72)**	**75 (45-136)**	**1.74 (1.56-1.96)**

No: number; M: male; F: female; yr: year; kg: kilogram; m: meter

Muscle echo intensities

Except for the rectus femoris muscle, echo intensity was significantly increased in all other muscles with the forearm flexors reaching the highest levels (Table 8.2). Moreover, if abnormal, the forearm flexors sometimes showed a typical pattern with predominant involvement of the flexor carpi ulnaris and flexor digitorum profundus while the flexor digitorum superficialis and flexor carpi radialis were largely spared (Figure 8.1). This distinct pattern was present in both ClCh and NaCh and visible in 15% of all patients.

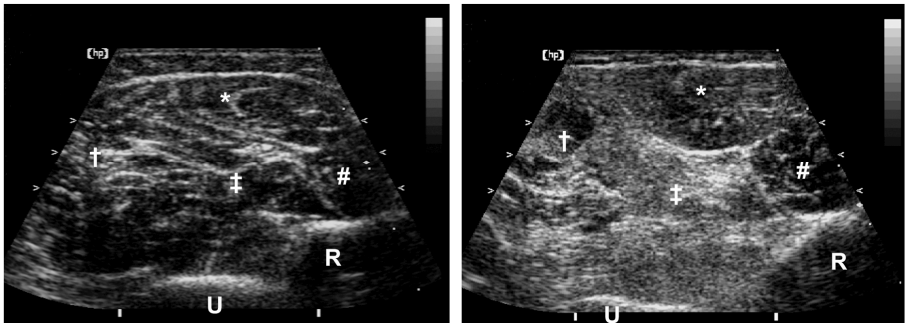

Figure 8.1 Transverse ultrasound image from the forearm flexors of a 56-year-old healthy woman (left-hand panel) and a same aged female patient with recessive myotonia congenita (right-hand panel). Comparison of the two images reveals a typical pattern within the forearm flexors of the patient, with predominantly higher echo intensities for the flexor carpi ulnaris (†) and flexor digitorum profundus (‡), and relatively spared flexor digitorum superficialis (*) and flexor carpi radialis (#). U: ulna; R: radius.

Table 8.2 The mean standardised muscle thickness and echo intensity readings (standard deviation) of the four muscles under investigation for all non-dystrophic myotonic syndromes together, for the two types of channelopathies separately, and for male and female patients separately.

Number of patients	NDMs (n=63)	ClCh (n=34)	NaCh (n=29)	Men (n=37)	Women (n=26)
Echo intensities					
Left biceps brachii	1.33 (1.6)*[1]	1.36 (1.5)*[1]	1.31 (1.6)*[1]	1.97 (1.6)*[1]	0.45 (1.0)*[2] $[2]
Right forearm flexors	2.17 (1.7)*[1]	2.77 (1.8)*[1]	1.47 (1.2)*[1] $[1]	2.55 (1.7)*[1]	1.63 (1.6)*[1] $[3]
Right rectus femoris	0.27 (1.2)	0.32 (1.2)	0.22 (1.5)	0.83 (0.9)*[1]	-0.50 (1.0)*[1]$[2]
Left tibial anterior	1.74 (1.1)*[1]	1.85 (1.1)*[1]	1.60 (1.0)*[1]	1.77 (1.0)*[1]	1.68 (1.2)*[1]
Muscle thickness					
Left biceps brachii	0.52 (1.2)*[2]	0.69 (1.1)*[2]	0.32 (1.3)	0.55 (1.3)*[2]	0.47 (1.1)*[2]
Right forearm flexors	0.85 (1.7)*[1]	0.53 (1.6)	1.23 (1.6)*[1]	0.55 (1.9)	1.28 (1.2)*[1]
Right rectus femoris	-0.01 (1.3)	0.26 (1.1)	-0.32 (1.5)	0.01 (1.5)	-0.04 (1.0)
Left tibial anterior	-0.13 (0.7)	-0.21 (0.7)	-0.03 (0.7)	-0.13 (0.8)	-0.12 (0.7)

NDMs: non-dystrophic myotonic syndromes; ClCh: chloride channelopathies; NaCh: sodium channelopathies; *[1] Significantly elevated (p<0.0001) relative to norm scores; *[2] Significantly elevated (p<0.05) relative to norm scores; $[1] Significant difference (p=0.002) between chloride and sodium channelopathies; $[2] Significant difference (p<0.0001) between male and female patients; $[3] Significant difference (p<0.05) between male and female patients

Subgroup analysis

The echo intensities of the forearm flexors were significantly higher in the ClCh than they were in the NaCh (Table 8.2). Except for the tibialis anterior muscle, all echo intensities were also significant higher in the male patients (Table 8.2). The intensities of the dominant biceps brachii muscle and the dominant forearm flexor muscles were significantly higher (44.7±9.2 versus 39.9±9.5 /256 grey levels; p=0.002 and 40.1±8.6 versus 38.2±7.9/256 grey levels; p=0.038, respectively) than the levels observed for

the non-dominant side. In the healthy controls the reverse was true: the intensities for the dominant muscles were significantly lower (difference -2.8; p<0.001 and -1.3; p=0.018, respectively) than they were in the non-dominant ones.

Muscle thickness

The arm muscles were significantly more pronounced than the values observed in normal, healthy individuals (Table 8.2), whereas the results for the leg muscles fell in the normal range.

Subgroup analysis

In patients with a non-dystrophic myotonic syndrome there were no significant differences in muscle thickness between the ClCh and NaCh nor between men and women (Table 8.2). However, the dominant forearm flexors in non-dystrophic myotonic syndrome patients were significantly thicker (difference 0.17 cm; p<0.001) than the non-dominant forearm flexors. In our previously established data of healthy controls we recorded no such significant difference (only 0.1 cm). In contrast, the biceps brachii muscle normally is significant thicker at the dominant side (0.2 cm), whereas in non-dystrophic myotonic syndrome patients we did not detect a significant difference between the dominant and non-dominant thickness of the biceps brachii muscle.

Muscle forces and ranges of motion

Table 8.3 shows the mean muscle force values in Newton (standard deviation) and the ranges of motion in degrees (standard deviation) for all non-dystrophic myotonic syndromes together and for the ClCh and NaCh separately.

Table 8.3 The mean muscle force readings in Newton (standard deviation) and mean ranges of motion in degrees (standard deviation) for all non-dystrophic myotonic syndromes together and for the two types of channelopathies separately.

Number of patients	NDMs (n=63)	ClCh (n=34)	NaCh (n=29)
Muscle force			
Elbow flexion	89 (41)	84 (38)	95 (44)
Three-point grip	155 (58)	140 (53)	172 (60) $[1]
Knee-extension	ND	ND	ND
Foot dorsoflexion	125 (46)	118 (49)	134 (42)
Range of motion			
Elbow	113 (27)	106 (24)	121 (28) $[1]
Wrist	139 (15)	134 (16)	145 (13) $[1]
Knee	126 (12)	123 (12)	131 (11) $[1]
Ankle	61 (14)	57 (13)	66 (14)$[1]

NDMs: non-dystrophic myotonic syndromes; ClCh: chloride channelopathies; NaCh: sodium channelopathies; ND: Not done. $[1] Significant difference (p<0.05) between chloride and sodium channelopathies.

Correlations

Table 8.4 shows the correlations between the muscle echo intensity and range of motion, muscle force exerted and age for each muscle separately. The echo intensity levels of all muscles showed significant negative correlations with the corresponding ranges of motion and the intensity of the forearm flexors showed a significant negative correlation with muscle force as measured by the three-point grip. The echo intensities for the rectus femoris muscle were significantly positively related to age, as were the intensities for the sum score. Muscle thickness of the right forearm flexors showed a significant positive correlation with muscle force (r=0.44; p<0.001) and was negatively correlated with age (r=-0.33; p=0.009). The muscle thickness sum score was also negatively correlated to age (r=-0.23; p=0.035).

Table 8.4 Correlations between the echo intensities and corresponding ranges of motion, muscle force and age for the four muscles and rank sum z-scores. Correlation coefficients are given in r-values (p-value).

Echo intensity	ROM	Muscle force	Age
Biceps brachii muscle	-0.43 (<0.001)*	0.10 (0.46)	0.13 (0.31)
Forearm flexors	-0.47 (<0.001)*	-0.30 (0.02)*	0.23 (0.07)
Rectus femoris	-0.40 (0.001)*	ND	0.31 (0.02)*
Anterior tibial muscle	-0.27 (0.04)*	-0.13 (0.32)	-0.01 (0.93)
Sum z-score	-	-	0.22 (0.05)*

ROM: range of motion; ND: not done; * Significant correlation.

Discussion

To our knowledge, ours is the first study to systematically investigate muscle changes and muscle thickness in a large cohort of genetically confirmed patients with non-dystrophic myotonic syndromes and to correlate the obtained data to functional parameters. The results revealed that the patients showed high echo intensities in all muscles (except the rectus femoris), indicating muscle changes.[12,14,17,18] Furthermore, the arm muscles showed hypertrophy. The muscle echo intensities were negatively correlated to the patients' ranges of motion and to the muscle force in their forearm flexors. The changes were, moreover, positively correlated to age and more pronounced in the dominant arm muscles. Together, these data suggest structural muscle changes in non-dystrophic myotonic syndromes. Although the correlations were not very strong, muscle changes seem to increase with age and degree of muscle use.

The muscle echo intensities were negatively correlated with the corresponding ranges of motion. Since high echo intensities are caused by muscle changes such as fat and fibrosis, these processes may subsequently lead to muscle shortening, which will limit the range of motion.[1] The muscle changes we observed are thus more than likely responsible for a limited range of motion (contracture) in non-dystrophic myotonic

syndromes. This pathophysiological mechanism has also been described in other skeletal muscle diseases like Duchenne's and Emery-Dreifuss muscular dystrophy and Ullrich's syndrome.[30-33] The echo-intensity did not differ between ClCh and NaCh, except for the forearm flexors. The range of motion, however, was significantly lower in ClCh than in NaCh. If the decreased range of motion would be due to fibrosis, range of motion measurements might be more sensitive than ultrasound measurements as a measure of fibrosis. Future research projects should investigate this hypothesis.

The highest muscle echo intensities were found in the dominant forearm flexors of our male patients. Since myotonia is most common in the forearm flexors and more pronounced in men than in women, it can be hypothesised that the muscle changes in non-dystrophic myotonic syndromes are exacerbated by the duration of the exposure to the symptom.[34,35] The long-term increase of sodium influx or a prolonged high intracellular sodium concentration may induce muscle fibre damage with as consequence muscle changes, which may subsequently lead to functional changes in terms of weakness and contractures.[36,37]

The positive correlation we found between the muscle echo intensity sum score and age is another argument supporting the hypothesis that the muscle changes are stepped up in proportion to the length of exposure to myotonia. Obviously muscle echo intensity increases with age, possibly due to an age-related replacement of muscle tissue by fat and fibrous tissue.[12,38] By using z-scores we already corrected for the influence of age in our healthy control group. As we still detected a correlation in non-dystrophic myotonic syndrome patients between z-scores and age, it means that myotonia at older age is more associated with higher echo-intensities. Thus, it is likely that structural muscle changes are especially found in older patients with a NDM, which is supported by the literature where myopathies are mainly reported in patients over 65 years.[39,40]

Unexpectedly, our muscular ultrasonography readings showed hypertrophy of the arm muscles only. Except in a few patients, we did not detect any significant hypertrophy in the quadriceps and anterior tibial muscles. With a test-retest correlation of 0.98-0.99 and a 0.99 correlation with MRI, muscle ultrasound is a reliable method to measure muscle thickness.[15, 41,42] In the literature, dominant myotonia congenita is reported with generalised muscular hypertrophy, whereas in recessive myotonia congenita especially the thighs, gluteal, shoulder girdle and calf muscles were hypertrophied.[8] When the condition is severe, the neck and (upper) arm muscles may also be involved.[8] In our study hypertrophy was most pronounced in the biceps brachii muscles of the patients with ClCh, whereas in the patients with NaCh the forearm flexors were hypertrophied. However, ClCh yielded higher echo intensities in the forearm flexors than NaCh. As yet, we have no sound pathophysiological explanation for the differences between these groups.

The greatest ultrasound abnormalities were detected in the forearm flexors of ClCh and men. Since both these groups also have relatively the smallest forearm size, one could hypothesise that the increased echo intensity may result from atrophy.

However, previous studies have shown that atrophy, for example caused by disuse, does not significantly increase muscle echo intensity.[16,19] Furthermore, in ALS the increased echo intensity is more pronounced than the atrophy, indicating that the structural changes are responsible for the increased echo intensity and not the atrophy.[43]

Muscle imaging in non-dystrophic myotonic syndromes has, to date, only been applied in a few patients with paramyotonia congenita but the few available results do support our ultrasound findings indicating that muscle changes occur in non-dystrophic myotonic syndromes: the MRI's of the gastrocnemius muscle showed a bilateral symmetrically elevated signal intensity on T1- as well as on T2-weighted images, which were interpreted as fatty infiltration.[44] Future research in NDMs should compare muscle ultrasound with MRI, preferable together with muscle histology as the golden standard.

Previous ultrasound studies showed that increased echo intensity is strongly related to structural muscle changes.[12,14,17,18,25] However, we emphasise that muscle ultrasound hyperechogenicity is an aspecific feature, that might reflect both fatty infiltration and fibrosis as well. As stated before, muscle biopsies are necessary to establish the exact nature of the muscle pathology in non-dystrophic myotonic syndromes. Biopsies performed in previous studies revealed only minor histopathological abnormalities.[9-11,45] However, most of these biopsies were obtained from proximal muscles, especially the quadriceps muscle.[9-11,45] In our study, the quadriceps muscle was the least affected muscle. In future studies muscle biopsies in non-dystrophic myotonic syndromes should be obtained from other affected muscles or guided by skeletal muscle ultrasound.[46,47]

In conclusion and in contrast to previous assumptions, quantitative muscle features in NDMs suggest structural muscle changes that increase with age and degree of muscle use. Forearm flexors are most affected. Future research should focus on muscle biopsies of the muscles that are most affected to further delineate the extent and nature of these structural changes. If these studies will also demonstrate some degree of muscle dystrophy, the name non-dystrophic myotonic syndromes could be open to debate.

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Part IV

Conclusions

Chapter 9

Summary, general discussion and future perspectives

General discussion

Clinical features of non-dystrophic myotonic syndromes (NDMs) were already described exhaustively in the pre-genetic era. However, after DNA analysis had become available, important genotype-phenotype mismatches were reported and a new subtype of NDMs, called Potassium Aggravated Myotonias (PAM) or Sodium-Channel Myotonias (SCM), was defined. It became clear that there was a need for an overall redefinition of NDMs that would be based on their distinct genotypes. In the present thesis the results of a nationwide study with the aim to redefine the clinical phenotypes of genetically confirmed NDMs are reported. Besides describing the phenotypes of NDMs on the basis of their genetics, it was our aim to provide clinicians with rules of thumb for focused genetic testing and to devise a better strategy to detect genetic mutations. Moreover, we looked for ways to facilitate better circumstances for the development of double blind randomised clinical trials for the treatment of myotonia. We evaluated the existing literature on the subject, and subsequently studied the genotypes, clinical features, health status and muscle ultrasound readings of a group adults with clinical and electrophysiological evidence of NDMs. Our systematic cross-sectional study about various clinical aspects of NDMs was conducted using a standardised protocol in one of the largest genetically confirmed NDM cohorts including both ClCh and NaCh. In this final chapter the results of our investigations are summarised and put into context, ending with future perspectives.

Chapter 1 gave a general introduction to NDMs, describing the clinical features of NDMs as defined in the pre-genetic era, the pathophysiology with respect to chloride and sodium conductance disturbances of the sarcolemma, the implicated genes, ancillary investigations, differential diagnoses and the current treatment options.

Chapter 2 comprised a systematic review of the available drug treatments for myotonia, the first in its kind. The literature search yielded ten randomised controlled trials in patients with myotonia comparing active drug treatment to placebo or comparing active drug treatment to any other active drug treatment. The trials either involved double-blind or single-blind crossover studies and included a total of 143 patients of which 113 had myotonic dystrophy type 1 and 30 had myotonia congenita. The majority of the trials were of poor quality, with some studies examining both patients with a dystrophic myotonic syndrome (i.e., myotonic dystrophy type 1) and patients with a non-dystrophic myotonic syndrome (i.e., myotonia congenita). This precluded a reliable analysis of the results reported. Three small-scale crossover studies evaluating myotonia in myotonic dystrophy type 1 were of good quality and demonstrated significant effects of imipramine, clomipramine and taurine, respectively. Unfortunately, for the treatment of myotonia in NDMs we were unable to find valid, separate data. Due to the scarcity and poor quality of the trials, we

hence could not establish whether the tested drug therapies for myotonia were safe or effective. Our review prompted the conclusion that well-designed double-blind randomised controlled (multi-centre) clinical trials, testing the drug effects for the different dystrophic and non-dystrophic types of myotonia separately, are needed. In the case of crossover trials, a washout interval is recommended, while intention-to-treat analyses and appropriate presentation of the results are indispensable.

Given that no randomised controlled clinical trials for myotonia in NDMs is available, past and current treatment strategies have been based on selective case reports, results from myotonic dystrophy type 1 trials, clinical experience, and theoretical benefits.[1-3] But what explains this lack of sound Randomised Controlled Trials (RCTs) in NDMs? Firstly, randomised, placebo-controlled trials in NDMs are difficult to set up because they are likely to be underpowered because of the rarity of NDMs.[2,3] Secondly, phenotypic and genotypic heterogeneity, with the possibility of different treatment responses, necessitates the definition of subgroups in such trials, which in turn, limits the number of patients even further. Lastly, it is difficult to define clinical endpoints for RCTs in NDMs since, to date, widely accepted outcome measures for such trials are lacking.[2]

Evidently, there is a pressing need for soundly designed and adequately supported multicentre trials. In the US the Food and Drug Administration (FDA) last year pledged to fund – within the framework of the orphan products grand programme- a placebo-controlled trial of mexiletine for the treatment of NDMs, which was started in December 2008.[3] However, in rare diseases with subsequent low numbers of eligible patients like NDMs, a controlled, multiple crossover trial, implemented in a single form (the 'n-of-1 design') is a viable, attractive alternative.[3] In such trials the patient acts as his or her own control, with the periods in which the agent under study and its placebo are used being randomly switched to determine the compound's therapeutic effect.[3,4] Studies based on the 'n-of-1 design' are hence especially recommended when individual treatment responses in rare diseases like NDMs are being investigated.

But which outcome measures are most suitable for RCTs or multiple crossover studies? For the evaluation of myotonia, standardised clinical bedside tests (Chapter 6) provide adequate parameters. To identify, confirm and quantify myotonia, several dedicated techniques are already available. Recently developed devices to assess the hand muscles are, however, not commercially available yet.[5,6] To test leg muscles, we propose the trunk sway analysis method.[7] The technique consist two angular-velocity transducers mounted on the lower back of the patient that allow his/her gait and postural stability to be objectively assessed. The results obtained in ten patients with DNA-proven RMC showed that postural instability can be accurately determined during naturalistic gait and balance tasks. [7] The authors also found it a useful tool to

detect and quantify myotonia in leg muscles and to detect and quantify the warm-up phenomenon in leg muscles. It proved to be especially sensitive and responsive when applied during tandem walking (eight successive toe-to-heel steps).[7] However, the trunk sway analysis does not distinguish between the effects caused by the myotonia and those originating from transient paresis. At last, it will be a major challenge to design devices to detect and quantify myotonia and the warm-up phenomenon in the eyelid muscles.

For the evaluation of clinical weakness, repetitive nerve stimulation (RNS) studies as described by Fournier et al. and high-density surface EMGs as described by Drost et al. are useful techniques.[8,9] However, both methods first require correlation studies with relevant clinical parameters. As clinical endpoints health status, functional disability and social participation are also vital parameters. Based on the results of our study presented in Chapter 7 we propose the SF-36 and FAS for the assessment of these aspects. We will discuss the desired standardisation of clinically relevant outcome measures and methods to test the clinimetric soundness of these outcomes measures later on in this chapter.

Chapter 3 was dedicated to the outline of the thesis.

As a redefinition of the clinical phenotypes of NDMs requires genetically homogeneous groups of patients we performed a genetic study in all the 54 probands we managed to recruit within one year. In **Chapter 4** their genetic profiles were determined by analysing *CLCN1* and *SCN4A* in tandem. With our study protocol we first sought to evaluate the relevance of in tandem analysis of the two channelopathies. Our second aim was to identify the mutations implicated in these probands and to compose homogeneous cohorts segregated by ClCh and NaCh. We established *CLCN1* mutations in 32 families (59%) and *SCN4A* mutations in 22 families (41%), reflecting a high-level of mutation ascertainment. However, the yield of our mutation detection was 93%, with six cases not yielding a second mutation. The genetic tests of all six were negative for myotonic dystrophy type 2. As suggested by Deymeer et al, these six cases could theoretically be carriers of NDMs.[10]

Our approach yielded 13 novel *CLCN1* and three novel *SCN4A* mutations, demonstrating the relative heterogeneity of the disease in the Netherlands. The clinical evaluations and DNA analyses of all first-degree relatives with a novel mutation revealed that all but one of these novel mutations segregated with the disease. Finally, our investigations enabled us to select homogeneous groups of patients segregated by the two channelopathies. In conclusion, in tandem analyses of *CLCN1* and *SCN4A* afford a high level of mutation ascertainment and allow homogeneous groups of patients with either ClCh or NaCh to be composed. Compared to previous studies, the yield of our mutation detection was high.[11-13] Based

on these results, we suggest that analysing *CLCN1* and *SCN4A* in tandem has a great diagnostic potential, offering optimal conditions for genotype-phenotype studies.[14]

In **Chapter 5** we discovered three NDM patients with a genotype-phenotype mismatch. In the pre-genetic era the warm-up phenomenon was assumed to be a specific symptom of ClCh. The three patients we examined, however, had a generalised warm-up phenomenon in combination with the V445M sodium-channel mutation. These three cases hence illustrate that the warm-up phenomenon is not typical for ClCh but may also occur in NaCh. It also underscores the urgency of a redefinition of the clinical phenotypes of NDMs for both ClCh and NaCh.

In **Chapter 6,** we reported the results of a descriptive cross-sectional study using standardised interviews and clinical bedside tests in 62 genetically confirmed NDM patients (32 ClCh and 30 NaCh) with the aim to redefine the clinical phenotypes of NDMs in the Netherlands and to formulate rules of thumb for focused genetic testing of either *CLCN1* or *SCN4A*. Based on widely used neurological examination procedures, we determined the presence and nature of myotonia (action myotonia and/or percussion myotonia), the relaxation times, and the presence of the warm-up phenomenon, paramyotonia, and transient paresis in three different body regions (i.e. the eyes and the upper and lower extremities). In the standardised interviews, patients with ClCh reported a higher frequency of muscle weakness, the warm-up phenomenon, and problems with standing up quickly, running, and climbing stairs. The patients with NaCh more often reported an earlier onset, and higher frequencies of paradoxical, and painful myotonia. The bedside tests revealed a higher incidence and longer relaxation times of myotonia in the leg muscles for the ClCh group and in eyelid muscles for the NaCh cohort.[15] The incidence and duration of relaxation times in the hand muscles were similar in both channelopathy groups. Furthermore, transient paresis was observed in ClCh only and paradoxical myotonia in NaCh only (Figure 9.1). Multivariate logistic regression analyses subsequently allowed us to develop the following clinical guidelines for genetic testing in NDMs: if both eyelid myotonia and transient paresis are absent OR if transient paresis is present (regardless of the absence or presence of eyelid myotonia), a ClCh is most likely. Conversely, if eyelid myotonia is present and transient paresis absent, a NaCh is the most likely diagnosis (see Chapter 6, Table 6.5).
Looking at ClCh in more detail, we did not find any variables differentiating between RMC and DMC, although it needs to be noted that our sample comprises only two DMC patients, indicating an apparent but unexpected scarcity of DMC in the Netherlands. This uneven distribution rendered a comparison of our clinical data on DMC and RMC unreliable. However, during our studies Fialho et al. were also comparing RMC and DMC in the UK.[16] In contrast to our study, theirs included an unexpectedly high proportion of DMC patients (37%), which was probably due to a founder effect. This did enable their team to contrast the two congenital myotonias

reliably: generalised muscle hypertrophy, transient paresis, and depressed upper limb reflexes were strongly associated with RMC but rare in DMC. Severe myotonia or muscle weakness were also typical seen in RMC.[16]

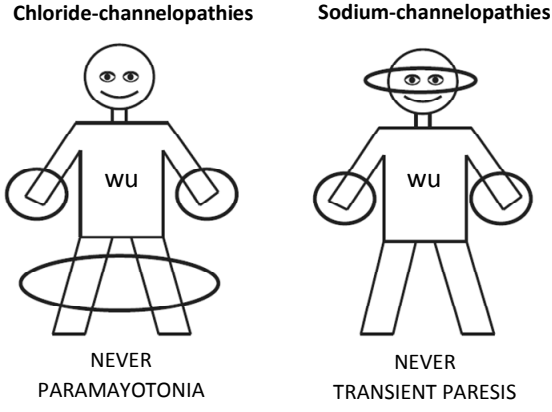

Figure 9.1 General clinical features in chloride (ClCh) and sodium channelopathies (NaCh). Patients with a ClCh especially show myotonia in the hand- and leg-muscles (ovals) and only a minority of these patients show myotonia in the eyelid-muscles. Patients with a ClCh never show paramyotonia. Patients with a NaCh especially show myotonia in eyelid- and hand-muscles (ovals) and a minority of these patients show myotonia in the leg-muscles. Patients with a NaCh never show transient paresis. Both forms of channelopathies may show the warm-up phenomenon (WU).

In our patients with a NaCh, the presence of myotonia in the leg muscles, and action myotonia and percussion myotonia in the right quadriceps muscle showed a trend towards the differentiation between SCM and PC, but it was only the warm-up phenomenon (SCM) that contributed statistically significantly to this differentiation.[15]

During our study two other research teams were also evaluating the clinical phenotypes of genetically confirmed NDM patients.[17,18] In the UK Matthews and colleagues studied clinical and genetic features in 32 patients with PC and in Canada Dupré et al. studied the clinical profiles of 50 genetically confirmed Canadian NDM patients.[17,18] In Matthews study, most PC patients mentioned cold, exercise or rest after exertion as exacerbating factors for their myotonia. The disease had first manifested itself in their early teens and especially affected the muscles of the face and upper limbs. The patients sometimes showed muscle weakness, especially in cold conditions or following exercise.[17] These findings accordingly confirmed von Eulenburg's original descriptions of PC. We observed the same symptoms in our Dutch group of PC patients.[15,19] Dupré et al. evaluated the clinical characteristics of 36 patients with *CLCN1* and 14 patients with *SCN4A* mutations.[18] In harmony with our study, they identified clinical features that were more likely to be associated with

particular genotypes. Their patients with *CLCN1* mutations, for example, typically showed myotonia in their lower extremities, and also suffered from myotonia of the tongue and from transient paresis. For their patients with *SCN4A* mutations, Dupré described eyelid myotonia and exacerbations of myotonia by cold temperatures as the leading symptoms. In accordance with our findings, they also found pain to be more frequent in this group of patients. They further noted that the warm-up phenomenon, hand- grip myotonia and percussion myotonia, the eyelid lag and hormonal effects did not differ between the two channelopathies.[18]

Besides the clinical features of NDMs, we were also interested in the impact on the patients' physical, psychological, and social functioning. These effects are evaluated in **Chapter 7**. Because NDMs are typically considered to be 'benign' diseases, little attention has been paid to its effects on the patients' ability to perform activities of daily living or their social participation.[20] Ours thus was the first study to determine the self-reported health status in patients with NDMs. In a cross-sectional design 32 patients with confirmed ClCh and 30 patients with confirmed NaCh, all off treatment, completed a standardised interview, the Fatigue Assessment Scale (FAS), and the 36-item Short-Form health survey (SF-36). We opted for the SF-36 because of its brevity, its extensive use in clinical studies, and its good psychometric properties.[21-24] The patients' SF-36 domain scores were compared with general community domain scores obtained in the Netherlands. We also conducted regression analyses to determine the strongest determinants of the SF-36 domains regarding general-health perception, and the physical component (PCS) and mental component summaries (MCS). All physically oriented SF-36 domains were substantially lower in both channelopathy groups compared to the Dutch community scores, reflecting a poorer clinical condition for the patients. In addition, 42% of the patients characterised their myotonia as painful and 53% could be classified as being fatigued. Fatigue proved to be the strongest predictor of low general-health perceptions, while painful myotonia best predicted low overall physical functioning. The level of social participation was also substantially lower in the NaCh patients compared to Dutch community scores, probably because of pain. None of the patients showed below-norm scores on the MCS domain. By showing that the impact of NDMs on the patient's health status is substantial and that particularly painful myotonia and fatigue tend to impede their physical functioning substantially, we disproved previous contentions that, given the condition's assumed mild nature, health-related aspects do not warrant particular attention. Because we were the first to use self-report questionnaires in this domain we also tested the validity and reliability of the scales used. Both the SF-36 and the FAS showed good content validity and reliability in our NDM cohort, making them potentially useful endpoints for future, randomised clinical trials.

In **Chapter 8** we systematically charted the changes in and thickness of four limb muscles from a large cohort of genetically confirmed NDM patients by means of

ultrasound and correlated the obtained data to functional parameters. The results showed elevated echo intensities in all muscles except the rectus femoris, and surprisingly, we only detected hypertrophy in the arm muscles. The elevated muscle echo intensities were significantly negatively correlated to the patients' ranges of motion and to the muscle force in their forearm flexors. The changes were positively related to age and more pronounced in the dominant arm muscles. Together, our readings suggest structural muscle changes in NDMs that increase with age and degree of muscle use. To establish the exact nature of the muscle pathology, muscle biopsies are required. In the past, such biopsies revealed only minor histopathological abnormalities,[25-27] but most were obtained from proximal muscles, especially the rectus femoris muscle.[25-27] However, this was the least affected muscle in our study. We propose that in future NDM studies muscle biopsies are obtained from the most affected muscles or guided by skeletal muscle ultrasound. However, the most affected muscles were the dominant forearm muscles and these are difficult to puncture, which implies that the procedure is not without risk, thus complicating further in-depth studies. If relevant muscle biopsies will be obtained in the future and if they will show structural (dystrophic) changes, the designation non-dystrophic myotonic syndromes would be open to debate.

As to the muscle hypertrophy we detected, it needs to be noted that the increased volume was restricted to the upper limb muscles whereas in the literature a definite generalised muscle hypertrophy is reported, particularly for RMC.[28,29] Also some of our patients showed clear generalised muscle hypertrophy, but others demonstrated a relative muscle atrophy. Using objective measures we thus established that in our NDM sample the mean muscle thickness of the patients' leg muscles was not enlarged.

What have we learned?

The Cochrane review in which we evaluated drug treatments for myotonia underlined that, to date, no double-blind randomised clinical trials have been conducted in NDM patients. In our genetic study we tested a new, approach to screen mutations in NDMs, i.e., in tandem analysis of *CLCN1* and *SCN4A*, which resulted in a 100% diagnostic yield. The technique, moreover, helped to uncover 13 novel *CLCN1* and three novel *SCN4A* mutations, expanding our knowledge of NDM subtypes. It also enabled us to compose genetically homogenous NDM groups on the basis of the two channelopathies, allowing us to describe their distinct clinical features and redefine the NDM phenotypes. Our investigations accordingly allowed us to develop new clinical guidelines for focused genetic testing. Moreover, it was possible to compare PC with SCM in NaCh, showing that only the warm-up phenomenon contributed statistically significantly to the differentiation between the two syndromes. Besides delineating the clinical features of NDMs in more detail, we were the first to study the

effects on the patients' physical, psychological and social functioning and found them to have a serious impact on the physical parameters. Furthermore, about 42% of our NDM patients reported painful myotonia and about 53% was fatigued. Using the SF-36 in combination with our standardised interviews and the FAS, painful myotonia proved the strongest predictor of the physical component summary and fatigue the strongest predictor of general-health perception. This new knowledge about the impact the various clinical features have on the patients' health status can be exploited to select outcome measures for future double-blind RCTs. Lastly, our muscle ultrasound measurements suggested structural muscle changes in NDMs, with hypertrophy being negligible and restricted to the upper limbs. The structural muscle changes preferably need to be confirmed by muscle biopsies.

What do we still need to know?

Numerous vital questions regarding genotype-phenotype relationships still remain unanswered, of course. For instance, how do different mutations relate to the specific phenotypic manifestations within and between kindred's with the same mutation?[1] And why do some individuals within an affected kindred manifest only mild or minimal symptoms while other members are severely affected?[1] Theoretically, these phenomena could possibly be explained by metabolic, hormonal or environmental influences or a combination of these factors. Myotonia, for instance, tends to intensify during pregnancy and menstrual periods,[15,22,30] while men with myotonia congenita have more severe symptoms than women.[15,31] However, the basis of these differences are, as yet, unknown and warrant further investigation. Recently, Fialho showed that both testosterone and progesterone may rapidly and reversibly inhibit wild-type ClC-1 channels expressed in *Xenopus* oocytes,[32] which may hence serve as an explanatory mechanism for the observed modulations in the severity of symptoms in myotonia congenita. Furthermore, ClC-1 channels *in vitro* can be inhibited via a rapid, non-genomic metabolic signalling pathway,[32] which phenomenon may potentially help provide a mechanistic explanation for the variable clinical observations in NDMs, meriting further scrutiny in humans. If confirmed, epigenetic factors may provide additional prospects for novel targeted therapies.

Future perspectives

Since clinical phenotypes can be misinterpreted, it is important to mention that phenotypic descriptions in the literature without proper genetic information should be considered with caution. For instance, a recently published paper in the journal of AAPOS (the American Association for Pediatric Ophthalmology and Strabismus) reported two cases with DMC in which orbital magnetic resonance imaging (MRI) showed extraocular muscle hypertrophy with pronounced delayed orbicularis relaxation after forceful blinking.[33] The authors first suggested these phenomena to be phenotypical characteristics of DMC, but subsequent genetic testing revealed no

disease-causing mutations in the 23 exons of the *CLCN1* gene.[33] This, of course, does not rule out DMC, but in the light of the findings we obtained in our study, we should strongly recommend to analyse *SCN4A*.[15,34] The authors did and actually they detect the V445M sodium-channel mutation in both patients.[35]

Before our approach can be used in diagnostic protocols, some of the results we obtained need to be confirmed in patient cohorts from other countries. During the 2008 annual meeting of the American Academy of Neurology (AAN) in Chicago, we were informed that such studies are currently underway in the US.[36] Furthermore, during the 2009 AAN meeting in Seattle, Meola et al. presented an Italian study which confirmed our results.[37] The American-based multicentre study will include very sizeable patient cohorts, which, besides the obvious advantages, probably also implies that the selected standardised clinical bedside tests for the various muscle groups will not be performed under the same conditions and almost certainly not by the same investigator, as was the case in the studies reported in this thesis. In such large-scale investigations, test-retests are highly recommended, which corroboration was logistically impossible in our studies, as well as inter-observer studies.

As stated, based on our phenotype redefinitions we were able to devise rules of thumb for focused genetic testing of either *CLCN1* or *SCN4A*. Although it was not our aim to define the clinical phenotypes of individual mutations in *CLCN1* or *SCN4A*, we were able to define a typical phenotype for the V445M sodium-channel mutation (Chapter 5). The definition of clinical guidelines for other individual mutations requires a far larger number of patients. Probably a combination of clinical bedside tests and ancillary investigations are more practicable. Fournier, for instance, recommends RNS studies to focus genetic testing.[8,38] Additionally, needle EMGs may detect differences in amplitude and frequency in the various mutations.[39] Although Fournier was not able to pinpoint apparent differences in the abundance of myotonic discharges between ClCh and NaCh[8,38], we did manage to identify different types of myotonic discharges for the two channelopathies in our patient cohorts (unpublished data). We hope future results will facilitate the development of needle EMG criteria for focussed genetic testing. It is even plausible that the combined data obtained by means of clinical bedside tests, nerve stimulation studies, and needle EMG will be superior in defining guidelines for targeted genetic testing.

Another interesting issue is the quantification of myotonia, a crucial element in improving our understanding of the natural history of the syndromes involved as well as for the evaluation of medication studies. Several techniques to quantify myotonia in the hand muscles have already been developed and tested.[5,40] The purpose of our study was not to quantify myotonia, but to describe its clinical pattern in three different body regions using true-to-life neurological tasks, to which end we developed standardised clinical bedside tests, all easily applicable in any outpatient

clinic. The tests indeed allowed us to discern a differentiating myotonic pattern for ClCh and NaCh. Ideally, for future double-blind RCTs, we should also be able to objectively quantify myotonia in the leg and eyelid muscles. For leg-muscle measurements we propose the trunk sway analysis elucidated earlier in this discussion.[7] Regrettably, techniques to detect and quantify myotonia in eyelid muscles are as yet lacking.

Maybe even more important for NDM research is the definition of sound clinical endpoints and outcome measures to facilitate double-blind RCTs. A first prerequisite is standardisation of the assessment of symptoms and signs. Moreover, the translation of these standardised, quantified symptoms and signs to the limitations the patients experience in their health status, should likewise be standardised. This is essential given earlier experiences with inflammatory neuropathies, where an overwhelming assortment of scales have been applied, thereby hampering any reliable comparison of the various trials.[41] Subsequently, the selected outcome measures need to be clinimetrically tested and need to be simple, communicable, valid, reliable, and responsive.[42-44] Moreover, all outcome measures should be unambiguously constructed and hence represent only one of the outcome levels as specified in the international classification of functioning, disability, and health (ICF) drafted by the World Health Organisation (WHO) and the WHO health status concept.[45-47] According to the ICF and the concept of health status, when designing an RCT, researchers should determine a priori outcome measures, i.e., which domains and outcome levels they intend to assess.[46,47] They should also incorporate the minimal clinically important difference (MCID), which has been defined as “the smallest difference in score in the domain of interest which patients perceive as beneficial and which would mandate [..] a change in the patient’s management”.[48] The MCID is not equivalent to statistical significance. In fact, results may show a statistical significance, as is generally seen in large samples, without providing an indication of clinical significance.[49] Sloan et al. have recently published a pragmatic review of existing practical guidelines for the assessment of the MCID.[50] In summary, one of the major challenges for future scientific research in NDMs will be defining accurate and viable outcome measures.

As a last recommendation, we would suggest scheduling a new ENMC (European Neuromuscular Centre) International workshop dedicated to NDMs. The last such workshop was in 1995 and concerned the topics of paramyotonia, potassium-aggravated myotonias, and periodic paralyses.[51] We think it is time that NDM experts from across the world exchange newly acquired insights and the latest developments in patient management practices. The possibility of setting up a large-scale, European, multicentre RCT with mexiletine should also be discussed, with priority being given to the inclusion/exclusion criteria and the required and potential sample sizes. Finally, a toolkit for future fundamental studies and double-blind RCTs should be developed to

help define patients, outcome measures, trial medications, and symptomatic treatment regimens for all NDM subtypes. Of course, the ultimate goal of future investigations will be to find effective, evidence-based treatments for the various NDM syndromes.

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Samenvatting

Samenvatting

In dit proefschrift worden de resultaten van een nationale studie beschreven waarin we de genetica, de klinische kenmerken, de gezondheidsstatus en spierecho-waarden van een groep volwassen patiënten met een bewezen niet-dystrofische myotonie hebben bestudeerd. Het hoofddoel was om het fenotype van de niet-dystrofische myotonieën op basis van homogene genetische groepen (*CLCN1* en *SCN4A*, respectievelijk chloor- en natrium-kanalopathieën) te herdefiniëren. Daarnaast was het doel om een betere strategie voor het vinden van de verantwoordelijke mutaties te ontwikkelen en om optimale omstandigheden voor toekomstig dubbelblind gerandomiseerde studies naar myotonie te creëren.

In **Hoofdstuk 1** wordt een introductie en een historisch perspectief betreffende de niet-dystrofische myotonieën gegeven. Als eerste worden de klinische kenmerken van de niet-dystrofische myotonieën in het pre-genetische tijdperk beschreven. Daarna worden de pathofysiologie, de ontdekking van de verantwoordelijke genen, de mogelijkheden voor aanvullend onderzoek, de differentiaal diagnose en de behandelingsmogelijkheden van de niet-dystrofische myotonieën besproken. Vervolgens worden de argumenten voor het schrijven van dit proefschrift beschreven.

Hoofdstuk 2 behandelt de resultaten van het door ons verrichtte systematische review betreffende de medicamenteuze behandeling van myotonie. Er werden in dit review tien gerandomiseerde gecontroleerde trials geïncludeerd waarbij het effect van een medicamenteuze behandeling versus placebo of versus een andere medicamenteuze behandeling werd onderzocht. De geïncludeerde trials waren allen dubbel- of enkelgeblindeerde cross-over studies waarbij in totaal 143 patiënten werden geïncludeerd. Bij 113 patiënten was de diagnose myotone dystrofie type 1 en bij 30 patiënten was de diagnose myotonia congenita vastgesteld. Over het algemeen waren de geïncludeerde studies van slechte kwaliteit. Daarnaast werd in een aantal studies zowel patiënten met myotone dystrofie als patiënten met myotonia congenita geïncludeerd. Hierdoor was het niet mogelijk om van alle gevonden studies de resultaten betrouwbaar te analyseren. Samengevat zijn er uiteindelijk drie kleine cross-over studies bij patiënten met myotone dystrofie type 1 geselecteerd die van goede kwaliteit zijn. Deze studies waarin respectievelijk de middelen imipramine, clomipramine en taurine werden onderzocht toonden allen een significante vermindering van de myotonie aan. Helaas kunnen we geen betrouwbare data voor de behandeling van myotonie ten aanzien van de niet-dystrofische myotonieën presenteren. Concluderend is het vanwege te weinig onderzoek en insufficiënte data onmogelijk om te bepalen of medicamenteuze therapie voor myotonie veilig en effectief is. Wij concludeerden dat nieuwe dubbel-blind gerandomiseerd gecontroleerde onderzoeken nodig zijn waarbij de studies goed opgezet dienen te worden en waarbij de verschillende myotone aandoeningen afzonderlijk onderzocht

dienen te worden. Aangezien in de meest gevonden cross-over studies geen *washout* interval werd gebruikt, raden wij in de toekomst bij dergelijke studies wel een *washout* interval aan. Bovendien zijn een *intention-to-treat* analyse en een goede presentatie van de resultaten noodzakelijk.

Hoofdstuk 3 betreft een uiteenzetting van de opzet van dit proefschrift.

In **Hoofdstuk 4** worden de resultaten van de genetische analyse van achtereenvolgens *CLCN1* en *SCN4A* bij 54 index patiënten (*probands*) besproken. Het doel van deze studie was ten eerste om de genetische karakterisering van de niet-dystrofische myotonieën in Nederland te optimaliseren en ten tweede om homogene groepen patiënten, gesplitst in chloor- en natrium-kanalopathieën, te selecteren. Als resultaat van deze studie werden bij 32 families (59%) *CLCN1* mutaties en bij 22 families (41%) *SCN4A* mutaties gevonden. Deze studie heeft dan ook geresulteerd in een diagnostische opbrengst van 100%. Aangezien er echter bij drie recessieve en bij drie sporadische patiënten geen tweede mutatie werd gevonden was de opbrengst van individuele mutatie detectie 93% (*worst case scenario*). Onder alle gevonden mutaties ontdekten we 13 nieuwe *CLCN1* en drie nieuwe *SCN4A* mutaties. Klinische evaluatie en DNA-analyses van alle eerstegraads familieleden van patiënten met een nieuwe mutatie wees uit dat behoudens één mutatie alle andere mutaties met de ziekte segregeerden. Wij concludeerden dat de genetische analyse van achtereenvolgens *CLCN1* en *SCN4A* een zeer hoge mutatie detectie oplevert en dat het eveneens resulteert in de mogelijkheid om relatief homogene groepen patiënten, gesplitst in chloor- en natrium-kanalopathieën, te selecteren. Dit laatste gaf ons de mogelijkheid om de klinische kenmerken van de niet-dystrofische myotonieën in Nederland te herdefiniëren.

Hoofdstuk 5 beschrijft drie patiënten met een genotype-phenotype mismatch. In het pre-genetisch tijdperk werd namelijk verondersteld dat het warm-up fenomeen een specifiek symptoom was voor chloor-kanalopathieën. Wij beschrijven hier echter drie patiënten met een evident gegeneraliseerd warm-up fenomeen op basis van een V445M natriumkanaal mutatie. Wij concludeerden dat deze case-reports laten zien dat er een noodzaak is om de klinische kenmerken van de niet-dystrofische myotonieën te herdefiniëren.

In **Hoofdstuk 6** presenteren wij de resultaten van een descriptieve cross-sectionele studie waarin bij 62 genetisch bewezen patiënten met een niet-dystrofische myotonie, 32 chloor- en 30 natrium-kanalopathieën, gestandaardiseerde interviews en gestandaardiseerde klinische bedside tests werden afgenomen. De resultaten van de gestandaardiseerde interviews tonen dat patiënten met een chloor-kanalopathie vaker spierzwakte, het warm-up fenomeen en problemen bij snel opstaan, rennen en traplopen rapporteren. Patiënten met een natrium-kanalopathie rapporteren vaker

een vroeger begin van de klachten en gaven eveneens aan dat ze vaker last hadden van paradoxe en pijnlijke myotonie. De gestandaardiseerde klinische bedside tests tonen bij patiënten met een chloor-kanalopathie een hogere incidentie en langere relaxatie tijden van myotonie in de beenspieren en bij patiënten met een natrium-kanalopathie een hogere incidentie en langere relaxatie tijden van myotonie in de ooglidspieren aan. Een transiënte parese komt alleen voor bij chloor-kanalopathieën en paradoxe myotonie komt alleen voor bij natrium-kanalopathieën. Multivariate logistische regressie analyse gaf ons de mogelijkheid om klinische vuistregels voor het gerichter aanvragen van genetisch onderzoek op te stellen. Het komt er op neer dat op basis van klinische uitkomstmaten geadviseerd kan worden om eerst het *CLCN1* of eerst het *SCN4A* gen te analyseren. De in dit hoofdstuk beschreven klinische uitkomstmaten voorspellen met een sensitiviteit van 91% en een specificiteit van 97% of de patiënt een chloor-kanalopathie heeft. Vice versa voorspellen de klinische uitkomstmaten met een sensitiviteit van 97% en een sensitiviteit van 91% of de patiënt een natrium-kanalopathie heeft. Concluderend hebben we de klinische kenmerken van de niet-dystrofische myotonieën in Nederland geherdefinieerd. Daarnaast kunnen de aan- of afwezigheid van enkele eenvoudig te testen klinische kenmerken clinici helpen bij het gerichter aanvragen van genetisch onderzoek. Echter, indien er bij het eerste geanalyseerde gen geen afwijkingen gevonden worden, dient ook het tweede gen onderzocht te worden.

Gezondheidsstatus onderzoek binnen de niet-dystrofische myotonieën was voor de verschijning van dit proefschrift nooit onderzocht. Een dergelijk door ons uitgevoerde studie wordt in **Hoofdstuk 7** beschreven. Het hoofddoel van deze studie was om te bepalen wat de zelf-gerapporteerde gezondheidsstatus van patiënten met niet-dystrofische myotonieën is, zodat de impact van het ziektebeeld op het gebied van fysiek, psychologisch en sociaal functioneren beschreven kan worden. In een cross-sectionele studie werden bij 32 bewezen patiënten met een chloor- en 30 patiënten met een natrium-kanalopathie, waarbij overigens geen van de patiënten medicatie voor de myotonie gebruikte, gestandaardiseerde interviews afgenomen en vulden alle patiënten de *Fatigue Assessment Scale* (FAS) en de *36-item Short-Form health survey* (SF-36) in. De domeinscores van de SF-36 ten aanzien van patiënten met een niet-dystrofische myotonie werden vergeleken met beschikbare SF-36 domeinscores van de Nederlandse bevolking. Bovendien werden regressie analyses uitgevoerd om zo de sterkste determinant van de SF-36 domeinen *general-health perception*, *physical component summary* (PCS) en *mental component summary* (MCS) te bepalen. De belangrijkste resultaten toonden dat de scores van alle fysiek georiënteerde SF-36 domeinen in zowel chloor- als natrium-kanalopathieën en sociaal functioneren in natrium-kanalopathieën significant lager (slechtere *health-status*) zijn in vergelijking met de overeenkomstige domeinscores van de Nederlandse bevolking. Regressie analyse toonde aan dat vermoeidheid de sterkste voorspeller was voor een lagere *general-health perception* en dat pijnlijke myotonie de sterkste voorspeller was voor

een lagere *physical component summary*. Geen van de patiënten toonde overigens verlaagde scores op de *mental component summary*. Wij concludeerden dat de impact van niet-dystrofische myotonieën op het fysiek functioneren substantieel is en dat vooral pijnlijke myotonie en vermoeidheid factoren zijn die het fysiek functioneren van patiënten met een niet-dystrofische myotonie negatief beïnvloeden.

In **Hoofdstuk 8** beschrijven we een studie waarin middels spierecho de skeletspier-intensiteit en skeletspier-dikte van patiënten met een niet-dystrofische myotonie werden onderzocht. Bij een afwijkende spierecho-intensiteit of afwijkende spierdikte werd gekeken of deze afwijkingen correleerden met de eveneens gemeten functionele spierparameters. De belangrijkste bevinding van deze studie betreft een verhoogde spierecho-intensiteit, welke in de literatuur gecorreleerd is aan spierfibrose en spiervervetting, in bijna alle gemeten spieren. Daarnaast vonden we hypertrofie in de armspieren en niet in de beenspieren. De spierecho-intensiteits-waarden waren omgekeerd gecorreleerd met de bewegingsmogelijkheden van het overeenkomende gewricht en de spierecho-intensiteit was positief gecorreleerd met de leeftijd. Daarnaast waren de gevonden spierecho-intensiteitsafwijkingen ernstiger aan de dominante zijde. De spierecho-intensiteit van de voorarm-flexoren was omgekeerd gecorreleerd aan de spierkracht. Concluderend kan gesteld worden dat kwantitatieve spiermetingen bij patiënten met een niet-dystrofische myotonie structurele spierveranderingen suggereren. Deze structurele spierveranderingen zijn gecorreleerd aan de mate van spiergebruik. Om de histopathologische origine en de mate van aantasting van het spierweefsel verder te onderzoeken dient toekomstig onderzoek zich te richten op het afnemen van spierbiopten in de meest aangedane spieren of dienen echo-geleide spierbiopten afgenomen te worden. Indien deze studies eveneens een zekere mate van spierdystrofie aantonen, dan kan de naam niet-dystrofische myotonieën ter discussie worden gesteld.

Concluderend hebben we in dit proefschrift middels een review aangetoond dat er geen dubbelblind gerandomiseerde studies naar de behandeling van myotonie bij de niet-dystrofische myotonieën zijn uitgevoerd. Onze genetische studie toont aan dat het achtereenvolgens testen van *CLCN1* en *SCN4A* tot een zeer hoge mutatie-detectie leidt en dat deze methode helpt bij het samenstellen van homogene genetische groepen. Verder beschrijft dit proefschrift de klinische herdefinitie van de niet-dystrofische myotonieën. Hierbij beschrijven we klinische vuistregels voor het gerichter aanvragen van genetisch onderzoek. Daarnaast blijkt dat de impact van de ziekte op het dagelijks leven groter is dan wellicht werd gedacht. Tevens hebben we door een beter klinisch inzicht te krijgen betere omstandigheden gecreëerd voor toekomstig op te zetten dubbelblind gerandomiseerde klinische trials voor de behandeling van myotonie. Als laatste suggereren spierecho studies dat er bij patiënten met niet-dystrofische myotonieën waarschijnlijk toch structurele spierafwijkingen ontstaan.

Uiteindelijk bediscussiëren we in **Hoofdstuk 9** de resultaten van dit proefschrift. Als laatste hebben we in dit hoofdstuk de toekomstige mogelijkheden voor wetenschappelijk onderzoek op het gebied van de niet-dystrofische myotonieën beschreven.

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Dankwoord

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Curriculum vitae

Curriculum vitae

Jeroen Trip werd op 25 september 1975 geboren in Veendam. Van 1987 tot 1993 volgde hij het VWO aan de Rijksscholengemeenschap Winkler Prins te Veendam. Na afronding hiervan studeerde hij Geneeskunde aan de Rijksuniversiteit Groningen. In 1998 werd het doctoraal examen met goed gevolg afgelegd. Na het doctoraal volgden de co-schappen in het Academisch Ziekenhuis Groningen. Het keuze-coschap neurologie werd onder leiding van Dr. G. de Jong gevolgd in de Isala klinieken, locatie de Weezenlanden, te Zwolle. Aansluitend werd in 2000 het arts-examen cum laude behaald. Zijn medische carrière begon hij daarna als AGNIO op de afdeling neurologie van de Isala klinieken. De opleiding tot neuroloog volgde hij in het Academisch Ziekenhuis Maastricht met als opleiders Prof. dr. J. Troost, Prof. dr. M. Limburg en Dr. M.C.T.F.M. de Krom. Gedurende deze opleiding kreeg hij door middel van een subsidie van het Prinses Beatrix Fonds de ruimte voor het verrichten van klinisch wetenschappelijk onderzoek. Dit onderzoek, dat een samenwerkingsverband met de afdeling Neurologie van het Universitair Medisch Centrum St Radboud te Nijmegen betrof, heeft dit proefschrift als resultaat. Een deel van dit promotieonderzoek werd bekroond met de Neurologie Jaarprijs 2007 van de Nederlandse Vereniging voor Neurologie en de Pelerinprijs 2007 van het Academisch Ziekenhuis Maastricht. Per 1 december 2009 is Jeroen begonnen als neuroloog in het Diaconessenhuis te Meppel.

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List of publications

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Glossary

Glossary

Acetazolamide responsive myotonia congenita: potassium sensitive form of myotonia with painful myotonia, which responds to acetazolamide

BP: body pain

CI: confidence interval
ClCh: chloride channelopathies (DMC and RMC)
CMAP: compound muscle action potential
CLCN1: skeletal muscle chloride channel gene, mapped to chromosome 7q35

DM1: myotonic dystrophy type 1
DM2: myotonic dystrophy type 2 (PROMM)
DMC: dominant myotonia congenita

Exercise-induced delayed onset myotonia: myotonia appearing after a prolonged period of exercise, which is a clinical phenomenon of myotonia fluctuans
EMG: electromyography

FAS: fatigue assessment scale

GPH: general-health perception

HyperPP: hyperkalemic periodic paralysis
HYPP: hyperkalemic periodic paralysis with myotonia

MC: myotonia congenita
MCS: mental component summary
MH: mental health
MRC: medical research council, range 1-5
MVC: maximum voluntary contraction
Myotonia: clinical phenomenon, which refers to delayed muscle relaxation after voluntary or evoked muscle contraction. It is experienced by patients as muscle stiffness
Myotonia fluctuans: Potassium sensitive form of NDM with fluctuations of myotonia and exercise-induced delayed onset myotonia
Myotonia permanens: postassium sensitive form of NDM with serious and continuous myotonia

NaCh: sodium channelopathies (PAM or SCM and PC)
NDMs: non-dystrophic myotonic syndromes: skeletal muscle disorders including chloride (myotonia congenita) and sodium channelopathies (potassium aggravated myotonias or sodium-channel myotonias, paramyotonia congenita and hyperkalemic periodic paralysis with myotonia
NNH: number needed to harm
NNT: number needed to treat

NRS: numerical rating scale, range 1-10

PAM: potassium aggravated myotonia (myotonia fluctuans, myotonia permanens, acetazolamide responsive myotonia congenita)
Paramyotonia: paradoxical myotonia, i.e. worsening of myotonia after repetitive muscle contractions
PC: paramyotonia congenita
PCS: physical component summary
PhF: physical functioning
PROMM: proximal myotonic myopathy (DM2)

RCT: randomised controlled trial
RFE: role functioning emotional
RFPh: role functioning physical
RMC: recessive myotonia congenita (M. Becker)
RNS: repetitive nerve stimulation

SCM: sodium-channel myotonias: PAM diagnosed without a potassium loading test
SCN4A: skeletal muscle sodium channel gene, mapped to chromosome 17q 23-25
SD: standard deviation
SF: social functioning
SF-36: 36-item short-form health survey

Transient paresis: transient decline in muscle force

Vit: vitality

Warm-up phenomenon: Improvement of myotonia and transient paresis after repetitive muscle contractions
WMD: weighted mean difference

STELLINGEN

behorende bij het proefschrift

REDEFINING THE NON-DYSTROPHIC MYOTONIC SYNDROMES

Phenotypic characterisation based on genetic testing

Jeroen Trip

Nijmegen, 22 januari 2010

1. Volgens de 'evidence based medicine' is er geen klasse I bewijs ten aanzien van de medicamenteuze behandeling voor myotonie. (dit proefschrift)

2. Door bij een groep patiënten met een niet-dystrofische myotonie op basis van geherdefinieerde klinische kenmerken het *CLCN1*-gen dan wel het *SCN4A*-gen op aanwezige mutaties te analyseren wordt de diagnostische opbrengst sterk vergroot. (dit proefschrift)

3. Voor het onderscheid tussen chloor- en natrium-kanalopathieën heeft het warm-up fenomeen geen diagnostische waarde. (dit proefschrift)

4. Chloor-kanalopathieën hebben een hoge incidentie en een lange relaxatietijd van myotonie in de beenspieren. Natrium-kanalopathieën daarentegen hebben een hoge incidentie en een lange relaxatietijd van myotonie in de ooglidspieren. (dit proefschrift)

5. De impact op het fysiek functioneren van patiënten met een niet-dystrofische myotonie is substantieel. Pijnlijke myotonie en vermoeidheid zijn factoren die dit fysiek functioneren in negatieve zin beïnvloeden. (dit proefschrift)

6. Spierecho-intensiteitswaarden en functionele spiermetingen bij een groep patiënten met een niet-dystrofische myotonie suggereren structurele spierveranderingen. Indien spierbiopten dit bevestigen, dan kan de naam niet-dystrofische myotonieën ter discussie worden gesteld. (dit proefschrift)

7. Patiënten met een niet-dystrofische myotonie kunnen in een warenhuis beter niet de roltrap nemen.

8. La médicine, c'est guérir porfois, souloger souvent, consoler toujours.
   De geneeskunde, dat is soms genezen, vaak verlichten, altijd troosten.
   (Ambroise Paré)

9. Wie behouden wil zal steeds verliezen en wie bereid is te verliezen, zal behouden ook al behoudt hij iets heel anders dan hij zich oorspronkelijk had voorgesteld.

10. Kritiek krijgen betekent ook dat iemand belangstelling voor je heeft. (Ton Boot)